15 Extreme temperatures in the adult stage shape delayed effects of larval pesticide stress: a
16 comparison between latitudes
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**Abstract**

Global warming and pesticide pollution are major threats for aquatic biodiversity. Yet, how pesticide effects are influenced by the increased frequency of extreme temperatures under global warming and how local thermal adaptation may mitigate these effects is unknown. We therefore investigated the combined impact of larval chlorpyrifos exposure, larval food stress and adult heat exposure on a set of fitness-related traits in replicated low- and high-latitude populations of the damselfly *Ischnura elegans*. Larval pesticide exposure resulted in lighter adults with a higher water content, lower fat content, higher Hsp70 levels and a lower immune function (PO activity). Heat exposure reduced water content, mass, fat content and flying ability. Importantly, both stressors interacted across metamorphosis: adult heat exposure lowered the reduction of fat content, and generated a stronger decrease in PO activity in pesticide-exposed animals. Larval pesticide exposure and larval food stress also reduced the defense response to the adult heat stress in terms of increased Hsp70 levels. In line with strong life history differences in the unstressed control situation, high-latitude animals were less sensitive to food stress (body mass and water content), but more sensitive to pesticide stress (development time and PO activity) and heat exposure (PO activity and Hsp70 levels). While low-latitude adults could better withstand the extreme temperature as suggested by the weaker increase in Hsp70, heat exposure similarly affected the delayed effects of larval pesticide exposure at both latitudes. Our study highlighted two key findings relevant for ecological risk assessment under global warming. Firstly, the delayed effects of larval pesticide exposure on adult damselflies depended upon subsequent adult heat exposure, indicating that larval pesticide stress and adult heat stress interacted across metamorphosis. Secondly, low- and high-latitude animals responded differently to the imposed stressors, highlighting that intraspecific evolution along natural thermal gradients may shape sensitivity to pesticides.

Keywords: carry-over effects; chlorpyrifos; damselfly; heat; latitudinal gradient; multiple stressors.

## Introduction

High temperatures are receiving increased attention in ecotoxicology as these typically increase the toxicity of contaminants such as pesticides (Noyes et al., 2009) and therefore are critical when extrapolating toxicity tests across seasons and along thermal gradients (Clements et al., 2012). Especially the need to understand the impact of pesticides under global warming generated a surge of studies (Landis et al., 2013; Moe et al., 2013). These studies generated very valuable information for the development of a predictive framework for ecological risk assessment along natural thermal gradients and under global warming.

Studies on global warming typically exposed organisms to a moderate temperature increase, as predicted by relevant warming scenarios (IPCC, 2007). Such moderate temperature increases are often beneficial for temperate organisms in the absence of contaminants (Deutsch et al., 2008; Stoks et al., in prep.). Global warming will, however, also be characterized by more frequent temperature extremes (IPCC, 2012) and these are more likely to negatively impact organisms (Jentsch et al., 2007). Yet, temperature extremes received much less attention in ecotoxicology (but see Kaur et al., 2011).

While most studies simultaneously exposed organisms to contaminants and warming, there is increasing concern that stressors may interact in a delayed way (Segner, 2011). Such delayed effects are easily overlooked in the many animals that have a complex life cycle with a larval aquatic stage and an adult, terrestrial stage, separated by metamorphosis (Moran, 1994). Several studies have documented carry-over effects of larval pesticide exposure on adult traits (e.g. Campero et al., 2008; Distel and Boone, 2010; Boone et al. 2013), but few

have explored how exposure to contaminants in the larval stage affects the sensitivity of the animals to a different stressor in the adult stage (e.g. Rohr and Palmer, 2005) and no studies so far did this in a global warming framework and tested how delayed effects of larval pesticide exposure are shaped by adult exposure to extreme temperatures.

Another aspect that may further increase realism to assess the impact of contaminants under global warming is the consideration of thermal adaptation shaping the sensitivity to contaminants at higher temperatures (Moe et al., 2013). Given that temperature extremes are more frequent at lower latitudes (Orlowsky and Seneviratne, 2012) it is to be expected that low-latitude populations may suffer less from extreme temperatures (Hoffmann et al., 2002). This generates the hypothesis that temperature extremes will differentially affect the carry-over effects of larval pesticide stress in low- and high-latitude populations.

We addressed all three above-mentioned issues by investigating the combined impact of larval sublethal pesticide exposure and subsequent adult exposure to extreme temperatures on a set of fitness-related traits in a common garden experiment with replicated populations at the lower and higher part of the latitudinal range of an aquatic insect. Given their limited ability to escape exposure, many ectothermic aquatic organisms are particularly vulnerable to combinations of contaminants and high temperatures (Bronmark and Hansson, 2002; Woodward et al., 2010). We additionally manipulated food stress in the larval stage, because this may make energy-mediated carry-over effects more apparent (Karl et al., 2011). Moreover, suboptimal food conditions may increase the impact of pesticide stress (Janssens and Stoks, 2013a) and of exposure to extreme temperatures (Adamo et al., 2012). As study species we chose the damselfly *Ischnura elegans* and tested animals originating from different latitudes, hence with a different evolutionary history of heat extremes. Damselflies are especially sensitive to global warming (Hassall and Thompson, 2008) and given their aquatic larval stage and terrestrial adult stage, stressor effects after metamorphosis may couple

aquatic and terrestrial ecosystems (Stoks and Cordoba-Aguilar, 2012). We quantified the following fitness-related traits in damselflies (Stoks and Cordoba-Aguilar, 2012): survival, emergence success and development time in the larval stage and flying ability, body mass, water content, fat content, levels of the stress protein Hsp70 and phenoloxidase activity in the adult stage. As model pesticide we chose chlorpyrifos, an organophosphate insecticide that functions as an inhibitor of acetylcholinesterase (Stenersen, 2004). Chlorpyrifos is one of the most used pesticides worldwide (Eaton et al., 2008) and is often found in ponds where it negatively affects non-target organisms, including aquatic insects (Rubach et al., 2012).

## 1. Methods

### *1.1 Collecting and housing*

We studied populations of the damselfly *Ischnura elegans* from two latitudes representing the low-latitude (southern France) and high-latitude (southern Sweden) parts of the range in Europe (Gosden et al., 2011). At each latitude three randomly chosen populations were sampled, namely Saint-Martin de Crau (+43°37'57.88"N, +4°46'55.18"E), Laune des Irudes (+43°30'34.80"N, +4°48'03.83"E) and Bassin de Réaltor (+43°28N11.16, +5°19'44.16"E) for France and Kalmar Dämme (+56°40'9.84"N, 16°17'48.48"E), Svino (+56°41'4.32"N, +16°22'23.79"E) and Hougårdsdammarna (+57°15'1.78"N, +12°8'1.983E) for Sweden. These populations were chosen in natural areas without agriculture and it is therefore unlikely that they were exposed to pesticides (Coors et al., 2009). Furthermore, any local adaptation to pesticides would be unlikely in coenagrionid damselflies given their high levels of gene flow (Johansson et al., 2013). At both latitudes temperatures above 30 °C have been documented, yet such hot days are much more frequent in southern France compared to southern Sweden. While in southern France (weather station Tour-du-Valat) the average number of days with air

125 temperatures equal to or above 30 °C equaled 34 days per year during the period 1992-2008,
126 this average was only one day per year in southern Sweden (weather station Malmö).
127 Eggs of fifteen mated females per population were collected and transferred to the
128 laboratory in Belgium. One week after hatching, larvae were placed individually in 200 ml
129 cups. Throughout the experiment, larvae were reared in incubators at 20 °C and L:D 14:10
130 hours. Larvae were daily fed *ad libitum* with *Artemia* nauplii five days a week (average daily
131 dose = 1347 nauplii, SE = 102, $n$ = 15 daily doses, each dose collected on a different day).

132 *1.2 Pesticide concentration*

133 Based on a previous experiment, we used pulses of 2.0 µg/l chlorpyrifos, since this causes a
134 growth reduction and only limited mortality in *I. elegans* damselfly larvae (Dinh Van et al., in
135 prep.). This concentration may be very high in general, but not in agricultural water bodies
136 preferred by the study species (Dijkstra 2006). Here peak concentrations often exceed 100
137 µg/l due to runoff (e.g. Moore et al., 2002; Mazanti et al. 2003; Bernabo et al., 2011).We
138 prepared the chlorpyrifos solution starting from a stock solution with a concentration of 20
139 µg/ml chlorpyrifos (kept in the dark at 4°C). This stock solution was a 50 times dilution of
140 another stock solution containing 1 mg/ml chlorpyrifos dissolved in ethanol. The chlorpyrifos
141 concentration of the stock solution at the start and at the end (3 months later) of the
142 experiment was 1.000 mg/ml and 0.975 mg/ml, respectively. Samples were analyzed by the
143 independent research laboratory Lovap NV (Geel, Belgium) using gas chromatography in
144 combination with mass spectrometry. The initial chlorpyrifos concentration in the
145 experimental vials was 2.020 µg/l and after three days (just before renewal of the medium) the
146 concentration was lowered to 0.884 µg/l, indicating that although the chlorpyrifos
147 concentration fluctuated in time, the larvae were continuously exposed to the pesticide.
148 We used aerated dechlorinated tap water in the control treatment instead of a solvent
149 control since the amount of ethanol was only 2 µl/l exposure medium. A pilot experiment

showed that there was no difference in survival, growth and development time of the study species at this ethanol concentration (Janssens, unpublished results). Moreover, the lowest NOEC reported for aquatic invertebrates is >10,000 times higher than the ethanol concentrations used in the pesticide treatment (UNEP, 2004).

*1.3 Experimental setup*

To test for effects of larval pesticide exposure, larval food stress and adult heat exposure on survival, life history and physiological variables in *I. elegans* damselflies and compare those between two different latitudes, we set up a full factorial experiment with two latitudes (France versus Sweden, with three replicated populations per latitude) × two food regimes (high food level versus low food level) × three pesticide conditions (control, 16 days exposure, complete final instar exposure) × two adult heat conditions (20 °C versus 30 °C) giving a total of 24 treatment combinations. We set up 35 larvae per treatment combination (total of 840 larvae). Due to differential mortality, sample sizes per response variable differ among treatment combinations and are shown in the figures.

The day after the larvae molted into the final instar (= $F_0$ stage), they entered the experiment. We used two different food levels: animals were fed *Artemia* nauplii twice a day six days a week (high food) or three days a week (low food); the individual doses per feeding were as before the exposure period (1347 nauplii ± 102). We used two exposure durations to the pesticide: exposure during 16 days of the final instar (the shortest duration of the final instar) and exposure during the complete final instar. The fixed 16-d period guarantees an equal exposure duration of all animals across treatments and therefore allows testing for differences in susceptibility or toxicity (the effect of a chemical at a controlled exposure duration) among treatment combinations (Rohr et al., 2011). The treatment with exposure during the complete final instar reflects the scenario in nature where animals of different latitudes and at different food regimes have a different duration of the larval stage, which may

generate different exposure times to contaminants. Larvae were exposed in cups containing 100 ml of medium, which was renewed three times a week (static renewal experiment). The chronic exposure design reflects a realistic situation of runoff exposure in water bodies close to agricultural land where animals are exposed to several pesticide pulses per week during the growing season of the crops (Van Drooge et al., 2001).

The day after the adults emerged, half of them was exposed to heat (30 °C, 60-70 % humidity) for 24 h, the other half was kept at control conditions (20 °C, 60-70 % humidity) for 24h. The frequency with daily maximum temperatures exceeding 30 °C, is often used as an index to investigate changes in extreme temperature events (e.g. Beniston et al., 2007). We chose for a short pulse instead of a gradual change and longer exposure, since animals can physiologically acclimate to the latter (Kaur et al., 2011).

*1.4 Response variables*

We checked animals daily for survival and adult emergence. Animals were considered as survived when they were alive at emergence and emerged successfully (i.e. fully expanded their wings). Animals with not-fully expanded wings are unlikely to reach sexual maturity. Development time was calculated as the number of days between molting into the final instar and adult emergence. Two days after emergence (directly after the 24 h exposure at 20 °C or at 30 °C), each adult was weighed to the nearest 0.01 mg (electronic balance, Mettler Toledo® AB135-S), and the sex was determined. Additionally, we scored the flying ability (0: not able to fly, 1: able to fly) of each adult that emerged successfully in a small insectary (30 cm x 30 cm x 30 cm) by stimulating it by gently tapping on the abdomen. Afterwards, each adult was stored at -80 °C for physiological analyses.

To quantify the water content, an important trait in the terrestrial stage of arthropods to withstand arid conditions (Edney, 1977), and the lipid content, an important fitness-related trait in damselflies (Stoks and Cordoba-Aguilar, 2012), we followed the protocol of Swillen et

al. (2009). The thoraxes were placed individually in Eppendorf tubes and weighed to the nearest 0.1 µg using a microbalance (Thermo Cahn C-35) before and after being dried for 48 h at 60 °C. The water content was calculated as the difference between the wet mass and the dry mass and expressed as a percentage of the total wet mass. Fat was extracted by adding 1 ml dichloromethane (99%) to the Eppendorf tubes, which were shaken for 24 h on an automatic shaker. Thereafter, the thoraxes were dried for another 48 h at 60 °C and weighed. Fat content was quantified as the difference between the dry mass before and after fat extraction and expressed as a percentage of the total dry mass.

To quantify the levels of the stress protein Hsp70, which protects animals against heat stress (Sörensen et al., 2003), we used the western blot assay described in Slos and Stoks (2008). Shortly, we first homogenized the heads of the larvae in 80 µl phosphate buffer (PBS, 100 mM, pH 7.4) and measured the protein content of the samples using the Bradford method. Afterwards we diluted 30 µl of the head supernatant with 30 µl of Laemmli sample buffer and from this mixture a sample corresponding with 10 µg of protein was used for the analysis. The proteins were separated using SDS-polyacrylamide gel electrophoresis (PAGE). Afterwards, we used two antibodies to detect Hsp70, a monoclonal primary antibody (dilution 1:1500, anti-Hsp70 SPC-103D, Gentaur Europe, Kampenhout, Belgium) and an alkaline phosphatase-conjugated secondary antibody (dilution 1:1000, D0486, DakoCytomation, Glostrup, Denmark). We scanned the blotting membrane using the HP Scanjet 8270 and quantified the optical density of the stress protein bands using Image Pro Plus. Levels of Hsp70 were expressed as mean optical density (mOD) per µg protein. To correct for variation among blots, we ran on every blot a control sample of 1 µl Hela Cell Lysate (Heat shocked, Stressgen). Hsp70 levels were only quantified on a random subset of 12 larvae per treatment combination (total of 288 larvae).

We quantified the activity of phenoloxidase, a key component of an insect's immune system (Gonzalez-Santovo and Cordoba-Aguilar, 2012), based on the protocol of Stoks et al. (2006a). In a first step, we extracted the hemolyph by perfusing the thorax of the animals with 300 µl phosphate buffer (PBS, 100 mM, pH 7.4). A 384 microtiter plate was filled with 40 µl hemolymph and 5 µl of chymotrypsin (5 mg/ml mili-Q water) and the mixture was incubated for 5 minutes at room temperature. This way all of the present pro-enzyme proPO was converted into PO. In a final step, we added 20 µl L-DOPA substrate (10 mM in PBS). We measured absorbance at 490 nm during 60 minutes every 30 seconds at 30 °C. PO activity was determined as the slope of the linear part (between 1600 s and 2400 s) of the reaction curve. Measurements were run in duplicate per larva and the means were used for statistical analysis. Additionally, we measured protein content in the hemolymph sample using the Bradford method.

*1.5 Statistical analyses*

To test for effects of latitude of origin, larval pesticide exposure, larval food level, the adult heat treatment and their interactions on the binary response variables total survival and ability to fly we used separate ANOVAs with binomially distributed errors and the logit link function with the Glimmix procedure in SAS v9.3. To test for effects of latitude of origin, larval pesticide exposure, larval food level, the adult heat treatment and their interactions on the continuous variables dry mass, development time, water content and fat content we ran separate ANOVAs using the mixed procedure of SAS v9.3. When analyzing the PO activity and Hsp70 levels we used an ANCOVA with as covariates the protein content and body mass, and the optical density of the Hela band, respectively. We simplified the models by removing non-significant interactions and covariates. Population nested in latitude was included as a random factor. We also included sex in the models, but since it is no focal variable of interest and to keep the manuscript focused, we will only present its results in appendix 2. When a test

indicated a significant pesticide effect, we performed contrasts to investigate which of the three pesticide conditions differed from each other. The full models and the associated parameter estimates are presented in appendices 1 and 2 respectively. Means and standard errors per treatment combination for each response variable are given in appendix 3.

## 2. Results

### *2.1 Survival and flying ability*

Survival was lower in Swedish (52 %) than in French animals (91 %) ($F_{1, 4} = 80.76$, $p < 0.001$). No adults died during heat exposure, but afterwards the flying ability was lower ($F_{1, 551} = 23.38$, $p < 0.001$; 20°C: 90% able to fly, 30°C: 67% able to fly); this pattern did not differ between French and Swedish animals (Latitude x Temperature: $F_{1, 554} = 0.35$, $p = 0.56$). Food level, the pesticide nor interactions between the different stressors affected survival or flying ability.

### *2.2 Development time and mass at emergence*

Development time (duration final instar) was longer in Swedish animals ($F_{1,607} = 2311.54$, $p < 0.0001$) and in animals reared at the low food level ($F_{1,607} = 20.67$, $p < 0.0001$). Swedish larvae exposed to the pesticide during the complete final instar had a longer development time at the high food level and a shorter development time at the low food level compared to the control larvae and the larvae exposed to the pesticide for 16 days. This was not the case in French animals (Latitude x Food x Pesticide; $F_{2,607} = 4.13$, $p = 0.017$) (Fig. 1A-B).

French adults had a lower dry mass than Swedish adults ($F_{1,5.85} = 13{,}69$, $p = 0.011$) and adults reared at the low larval food level were lighter than those reared at the high larval food level ($F_{1,441} = 113.31$, $p < 0.0001$). This reduction in dry mass at the low food level was more pronounced in French adults (Latitude × Food: $F_{1,441} = 17.53$, $p < 0.0001$) (Fig. 1C-D). Adult dry mass was lower when larvae had been exposed to chlorpyrifos ($F_{2,441} = 2.66$, $p =$

0.071; contrast analyses: $p_{\text{control-16days}} = 0.039$, $p_{\text{control-complete F0}} = 0.028$). There was no effect of the duration of the exposure period to chlorpyrifos on the dry mass (contrast analysis: $p_{\text{16 days-complete F0}} = 0.88$). Dry mass tended to be lower in adults which were exposed to the heat treatment ($F_{1,441} = 3.71$, $p = 0.055$). This heat effect did not depend on latitude, food level or chlorpyrifos exposure (all $p < 0.34$) (Fig. 1C-D).

*2.3 Physiology*

Water content was higher in French adults than in Swedish adults ($F_{1,6.28} = 14.73$, $p = 0.0079$), and higher in French adults reared as larvae at the low food level than at the high food level (Latitude x Food: $F_{1,439} = 9.89$, $p = 0.0018$) (Fig. 2A-B). Animals that were exposed to chlorpyrifos during the complete final instar had the lowest water content ($F_{2,439} = 2.87$, $p = 0.058$; contrast analyses: $p_{\text{control-complete F0}} = 0.047$; $p_{\text{16 days-complete F0}} = 0.039$). There was no difference in water content between the control animals and animals exposed to the pesticide for 16 days ($p_{\text{control-16days}} = 0.91$) (Fig. 2A-B). Water content was lower in adults which were exposed to heat compared with the control animals ($F_{1,440} = 34.85$, $p < 0.0001$).

Fat content was lower in French adults than in Swedish adults ($F_{1,5.88} = 158.42$, $p < 0.0001$). Animals reared at the low food level had a lower fat content ($F_{1,424} = 385.26$, $p < 0.0001$). Exposure to chlorpyrifos resulted in a lower fat content ($F_{2,425} = 178.6$, $p < 0.0001$), regardless of the exposure duration ($p_{\text{16 days-complete F0}} = 0.90$). The pesticide-induced reduction of the fat content was more pronounced at the high food level (Pesticide x Food: $F_{2,424} = 32.18$, $p < 0.0001$) (Fig. 2C-D). Exposure to heat resulted in a lower fat content ($F_{1,425} = 157.41$, $p < 0.0001$), with a smaller reduction in adults that were exposed as larvae to the pesticide (Pesticide x Temperature: $F_{2,425} = 11.47$, $p < 0.0001$) (Fig. 2C-D).

Hsp70 levels were higher in Swedish adults ($F_{1,\,290} = 234.25$, $p < 0.001$) and lower in adults reared as larvae at the low food level ($F_{1,\,290} = 114.41$, $p < 0.001$). This decrease in Hsp70 levels at the low food level was more pronounced in the Swedish adults (Latitude ×

299 Food: $F_{1, 290} = 4.05$, $p = 0.045$) (Fig. 3A-B). Exposure to the pesticide resulted in higher
300 Hsp70 levels ($F_{2, 290} = 21.34$, $p < 0.001$), especially in animals reared at the high food level
301 (Food × Pesticide: $F_{2, 290} = 4.78$, $p = 0.0091$) (Fig. 3A-B). There was no effect of the exposure
302 duration (contrast analysis: $p_{16\ days\text{-}complete\ F0} = 0.85$). Hsp70 levels were higher in adults which
303 were exposed to heat ($F_{1, 290} = 48.16$, $p < 0.001$). This heat-induced increase in Hsp70 levels
304 was more pronounced in Swedish adults (Latitude × Temperature: $F_{1, 290} = 12.18$, $p < 0.001$),
305 in animals reared at the high food level (Food × Temperature: $F_{1, 290} = 8.19$, $p = 0.0045$), and
306 in animals reared in the absence of the pesticide (Pesticide × Temperature: $F_{2, 290} = 3.09$, $p =$
307 0.047) (Fig. 3A-B).

308 PO activity was higher in Swedish adults ($F_{1,6.52} = 107.51$, $p < 0.0001$) and lower in
309 adults reared as larvae at the low food level ($F_{1,443} = 75.49$, $p < 0.0001$). Heat exposure caused
310 a decrease in PO activity, but only in Swedish animals (Latitude x Temperature: $F_{1,443} = 9.95$,
311 $p = 0.0017$) and in animals reared at the high food level (Food x Temperature: $F_{1,441} = 6.87$, $p$
312 $= 0.0091$). Chlorpyrifos exposure resulted in a lower PO activity ($F_{2,442} = 151.34$, $p < 0.0001$),
313 while there was no effect of the exposure duration ($p_{16\ days\text{-}complete\ F0} = 0.43$). The pesticide-
314 induced decrease in PO activity was the strongest in Swedish animals reared at the low food
315 level (Food x Pesticide x Latitude: $F_{2,441} = 6.01$, $p = 0.0027$) (Fig. 3C-D). The pesticide-
316 induced decrease in PO activity was most pronounced in animals reared at the low food level
317 and exposed to the heat (reduction of 53 %) and the least pronounced in animals reared at the
318 high food level without heat exposure (reduction of 32 %) (Food x Pesticide x Temperature:
319 $F_{2,442} = 3.96$, $p = 0.020$, Figure 3C-D).

320

321 **3. Discussion**

322 Exposure to the ecologically relevant chlorpyrifos concentration in the larval stage of *I.*
323 *elegans* affected nearly all sublethal traits scored after metamorphosis in the adult stage. As

shown in other studies, pesticide-exposed animals tended to be smaller at emergence (e.g. Janssens and Stoks, 2013a) and emerged as adults with lower energy reserves (e.g. Janssens and Stoks, 2013b) and lower immune function (e.g. Galloway and Handy, 2003). This can be explained by the investment in costly defence mechanisms in the presence of chlorpyrifos, including the here observed increase of Hsp70 (e.g. Janssens and Stoks, 2013b).

For most affected traits, the delayed effects of larval pesticide exposure on adult traits did not depend on the exposure duration. This suggests that longer exposure to the pesticide did not increase toxicity and that the animals were not able to recover (completely) from previous pesticide exposure, despite long durations of the larval post-exposure period in the 16-day exposure treatment (for example, the Swedish larvae that were exposed to the pesticide for 16 days had on average 56 days to recover before metamorphosis). Limited recovery at the individual level after pesticide exposure has been observed before (e.g. Rohr and Palmer, 2005) and is important when evaluating the effects of pesticides. This may not be surprising since the chosen chlorpyrifos concentration was already high compared to the $LC_{50\ 48h}$ for damselflies (5.28 µg/l, Brazner et al., 1988).

Only two traits were only affected when animals were exposed to the pesticide throughout the entire larval period: development time (see 4.2) and adult water content. We hypothesize the increased water content under long pesticide exposure to be a side-effect of an adaptive defense mechanism against pesticide uptake (Zhang et al., 2008), more specifically the reduction of the permeability of the cuticula by the incorporation of glycine-rich proteins (GRP) (Ahmad et al., 2006). The increase of GRP levels is energetically costly, what might explain the absence of a pesticide effect on the water content in animals that were only exposed for 16 days. The latter animals probably also decreased the permeability of the cuticula, but stopped GRP incorporation when the pesticide exposure ended.

### *3.1 Larval food stress and its interactions with pesticide stress*

Animals reared at the low food level showed the typical slower development and reduction in mass at emergence (Nylin and Gotthard, 1998; De Block and Stoks, 2005), and invested less in costly traits such as fat content (Rolff et al., 2004; Stoks et al., 2006b; De Block et al., 2008), immune function (Rolff et al., 2004; De Block and Stoks, 2008; Karl et al., 2011) and the stress protein Hsp70 (e.g. Muturi et al., 2011).

Food level and pesticide exposure interacted for fat content. The pesticide-induced reduction of fat was more pronounced at high food; probably because high-food larvae can afford a stronger decrease in fat and invested more in the costly Hsp70 defense.

### *3.2 Latitude-specific responses to pesticide stress and food stress*

We found strong phenotypic differentiation between the French and Swedish animals reflecting life history differences associated with latitudinal shifts in voltinism. The more favorable thermal conditions allow low-latitude populations to produce more than one generation per year, giving each generation, however, limited time to complete their life cycle thereby driving the evolution of a faster life history at low latitudes (e.g. Ragland and Kingsolver, 2007; for the study species: Shama et al., 2011; Stoks et al., 2012). This is here reflected in the shorter development times of the French larvae. A fast life history is, however, costly (Stoks et al., 2006b; Dmitriew, 2011) and is here traded off against a lower mass at emergence and a lower investment in Hsp70 (see also Stoks and De Block, 2011). We extended this fast-life phenotypic syndrome (*sensu* Réale et al., 2010) by also documenting lower investments in energy storage (fat content) and in immune function (PO activity). The higher investment in immune function in the Swedish animals in the absence of pathogens may explain their higher mortality (see Tucker and Stevens, 2003 for an example in beetles). The higher water content in French adults likely is an adaptation to the warm, dry conditions at low latitudes and is in line with the observation that insects adapted to hot and dry environments have a higher water content (e.g. Gibbs et al., 1997). A higher water content

374 may be achieved by a reduction of the permeability of the cuticula to prevent water loss by
375 the incorporation of glycine-rich proteins (Zhang et al., 2008). Alternatively, it is possible that
376 the French animals actively increased their water uptake to prevent emerging at a too small
377 size, since this will have important negative fitness consequences (e.g. lower fecundity)
378 (Stoks & Cordoba-Aguilar, 2012).

379 Despite strong latitudinal differences in nearly every variable in the unstressed control
380 situation, French and Swedish animals responded quite similar to the pesticide stress. Yet, for
381 two pesticide-affected variables, development time and PO activity, the French animals were
382 less sensitive. When exposed to the pesticide during the complete final instar, French larvae
383 did not change their development time, while Swedish larvae did so in a food-dependent way.
384 At high food, Swedish larvae delayed emergence probably because under optimal food
385 conditions this allowed them to compensate for the higher energy demands for detoxification
386 (Sibly and Calow, 1989; including the here observed higher Hsp70 levels). Yet, at low food
387 this was probably no option and they instead shortened the exposure period. Such adaptive
388 shortening of the larval stage to escape pesticide exposure has been observed before (e.g.
389 Distel and Boone, 2010; Rohr et al., 2011; Janssens and Stoks, 2013a). The absence of this
390 life history escape response in French larvae probably reflects a lower limit to their, already
391 very short, final instar (see also Stoks and De Block, 2011). Somewhat similar, the pesticide-
392 induced immunosuppression was stronger in Swedish adults reared as larvae at low food. As
393 we will argue for heat exposure, the high PO levels in Swedish animals may have relaxed the
394 need to buffer the pesticide-induced immunosuppression at low food. The smaller response in
395 French animals can be explained by their lower baseline PO levels that probably were close to
396 the admissible level to survive immune challenges (see also Campero et al., 2008).

397 In line with the observation that faster growing animals invest less in energy storage
398 and are more sensitive to food shortage (e.g. Stoks et al., 2006b), the food stress-induced

reduction in mass was stronger in French animals; this despite French adults already being lighter than the Swedish adults in the unstressed condition. In contrast, however, the food stress-induced reduction in Hsp70 was less strong in French animals, suggesting they tried to keep Hsp70 levels above a certain critical threshold, potentially to cope with future thermal stress. Only French animals increased their water content when reared at the low food level (for an example on collembolan, see Lavy et al., 1997). Adult size is fixed at emergence in insects and important for adult fitness (Stoks and Cordoba-Aguilar, 2012). We hypothesize that French animals reared at low food increased water uptake to compensate any size reductions due to the loss of mass and fat reserves.

*3.3 Adult heat exposure and its effect on carry-over effects of larval pesticide stress*

Heat exposure had strong negative effects on nearly every trait, suggesting it will negatively impact fitness. Besides the direct effect of a lowered water content (e.g. Atmowidjojo et al., 1997), heat-exposed animals suffered reductions in mass (e.g. Franke and Fischer, 2013), fat content and flying ability (e.g. Wang et al., 2009). This can be explained by the costly defense mechanisms against the heat treatment, including the here observed increase of Hsp70 levels (Sörensen et al., 2003). Additionally, heat exposure likely increased metabolic rate, thereby generating higher energy demands (Clarke and Fraser, 2004). Noteworthy, while it has been recently demonstrated that effects of an extreme temperature may be stronger at low food level (Adamo et al., 2012), this was not supported in our study.

In line with the much higher frequencies of extreme temperatures at their latitude, we found some indication that French adults could better deal with the extreme temperature. French adults responded with a weaker increase in Hsp70 in response to the heat treatment. We interpret this as the French larvae being more adapted to extreme temperatures, making it less necessary to rely on the energetically costly increase of Hsp70 (see e.g. Tomanek & Zuzow, 2010 for an example in mussels). Furthermore, only Swedish adults reduced PO

levels after the heat exposure. Yet, the latter effect may not necessarily reflect a lower ability to deal with heat stress, but rather the fact that Swedish adults can better afford a decrease in PO (given their higher levels in the unstressed controls). This may also explain why the heat-induced reduction in PO was only observed at high food. This adds to the few other studies showing heat-induced reductions in PO activity (Karl et al., 2011; Seppälä and Jokela, 2011), thereby indicating that extreme temperatures, which are predicted to increase in frequency under global warming (IPCC, 2012), may make animals more susceptible to pathogens.

A key finding was that adult heat exposure modulated the carry-over costs of the larval pesticide exposure, and this in opposite ways for fat content and PO activity. The pesticide-induced reduction in adult fat content was less strong in adults exposed to the heat treatment. This again can be interpreted as adults in treatment groups with higher values of a fitness-related trait (here fat content in adults not exposed to the heat treatment) can afford stronger reductions in the trait levels without going below a critical threshold value. In contrast, the pesticide-induced reduction in PO activity (at low food level) was stronger in heat-exposed adults. This can be explained as a physiological cost of the stress exposure: adults that were exposed to the pesticide during the larval stage, had probably a lower energy content which was invested in defense against the stressor that was present at that moment (i.e. heat) instead of investing it in long-term defense (i.e. immune response).

Larval pesticide exposure and larval food stress also reduced the defense response to the adult heat stress in terms of increased Hsp70 levels. This is likely the result of their lower energy content due to a lower energy income and/or need to invest in detoxification. This may also have contributed to the pattern of lowest immune function (PO activity) in heat-exposed adults that were exposed as larvae to a combination of pesticide and food stress.

### *3.4 Conclusions and implications for ecological risk assessment under global warming*

448 A key challenge for ecotoxicologists is to assess the impact of pesticides under global
449 warming (Noyes et al. 2009, Moe et al. 2013). By studying how carry-over effects of larval
450 pesticide exposure across metamorphosis are modulated by a subsequent adult heat exposure
451 in populations along opposite ends of a natural thermal gradient, our study highlighted two
452 novel key findings relevant for ecological risk assessment under global warming (Landis et
453 al., 2013). Firstly, larval pesticide stress and adult heat stress interacted across metamorphosis
454 making the delayed effects of larval pesticide exposure dependent upon subsequent adult heat
455 exposure for two fitness-related traits (fat and PO at low food). Related to this, animals
456 subsequently exposed to both stressors across metamorphosis suffered the lowest values of
457 these traits, making it relevant to consider these combined effects across metamorphosis when
458 predicting the fate of populations in polluted sites under global warming.
459 Secondly, in line with strong differentiation in the unstressed control situation, French
460 and Swedish animals responded differently to the imposed stressors. While the fast growing
461 French animals were more sensitive to larval food stress (for body mass and water content),
462 they were less responsive to the pesticide stress (for development time and PO activity) and
463 heat exposure (for PO activity and Hsp70 levels) compared to the Swedish animals. This
464 indicated that French suffered more under lower energy acquisition, while under an energy
465 allocation trade-off they better succeeded in maintaining minimum investment in immune
466 function and heat shock defense. These results highlight that intraspecific life history
467 evolution along natural thermal gradients may shape sensitivity to a pesticide and therefore
468 should be included in ecological risk assessment under global warming. More general our
469 results support the recent plea to include evolutionary developed phenotypic attributes when
470 considering the effects of contaminants in aquatic systems (Kamo et al., 2011; Segner, 2011).

471 **Acknowledgements**

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## Supplementary data

Appendix 1: Parameter estimates and their errors of the AN(C)OVAs testing for effects of latitude, food level, pesticide exposure and heat exposure on fitness-related traits in damselflies.

Appendix 2: Results of the full model AN(C)OVAs testing for effects of latitude, food level, pesticide exposure and heat exposure on fitness-related traits in damselflies.

Appendix 3: Means and their standard errors of the combined impact of latitude, food level, pesticide exposure and heat exposure on the continuous fitness related traits development time, dry mass, water content, fat content, Hsp70 levels and PO activity.

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687 **Figure legends**

688

689 **Fig. 1**: Mean development time (A, B) and dry mass (C, D) of *I. elegans* damselflies as a
690 function of latitude of origin, larval food stress, larval pesticide exposure and adult heat
691 exposure. Given are least-squares means ± 1 SE.

692

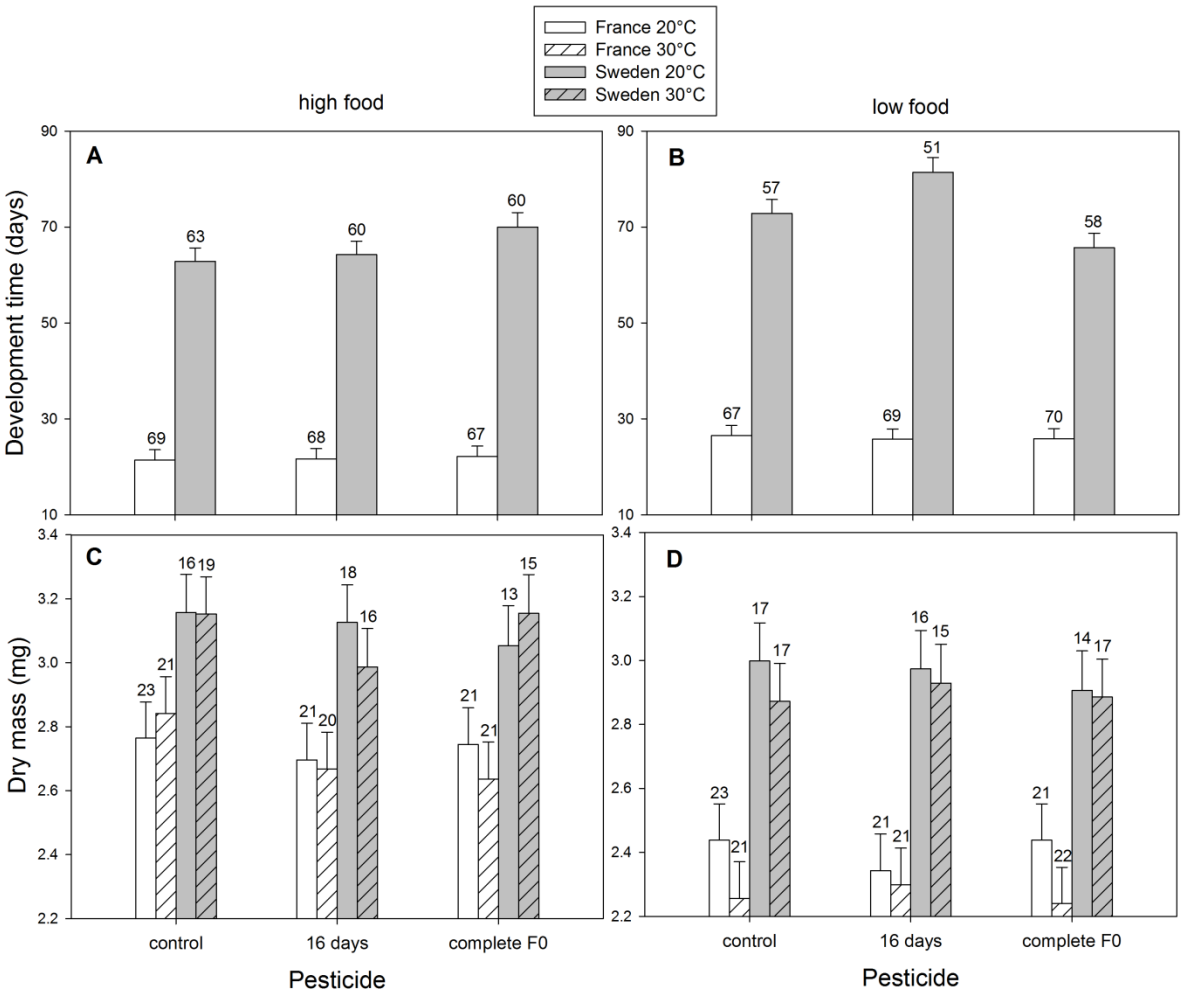
693 **Fig. 2**: Mean water content (A, B) and fat content (C, D) of *I. elegans* damselflies as a
694 function of latitude of origin, larval food stress, larval pesticide exposure and adult heat
695 exposure. Given are least-squares means ± 1 SE.

696

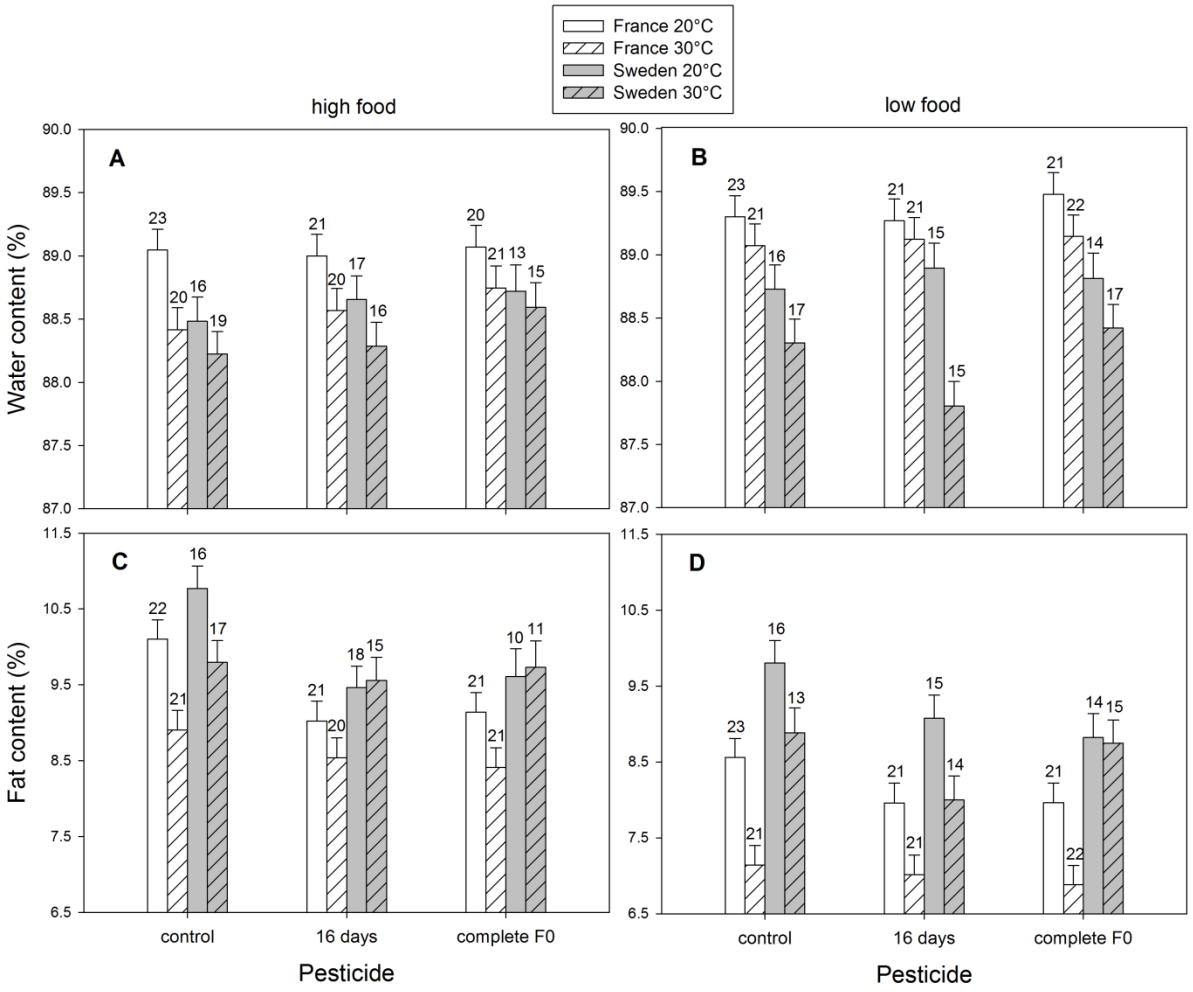
697 **Fig. 3**: Mean Hsp70 levels (A, B) and phenoloxidase activity (C,D) of *I. elegans* damselflies
698 as a function of latitude of origin, larval food stress, larval pesticide exposure and adult heat
699 exposure. Given are least-squares means ± 1 SE.

700

**Fig. 1**


France 20°C
France 30°C
Sweden 20°C
Sweden 30°C
high food
low food
A
B
C
D
Development time (days)
Dry mass (mg)
control
16 days
complete F0
Pesticide

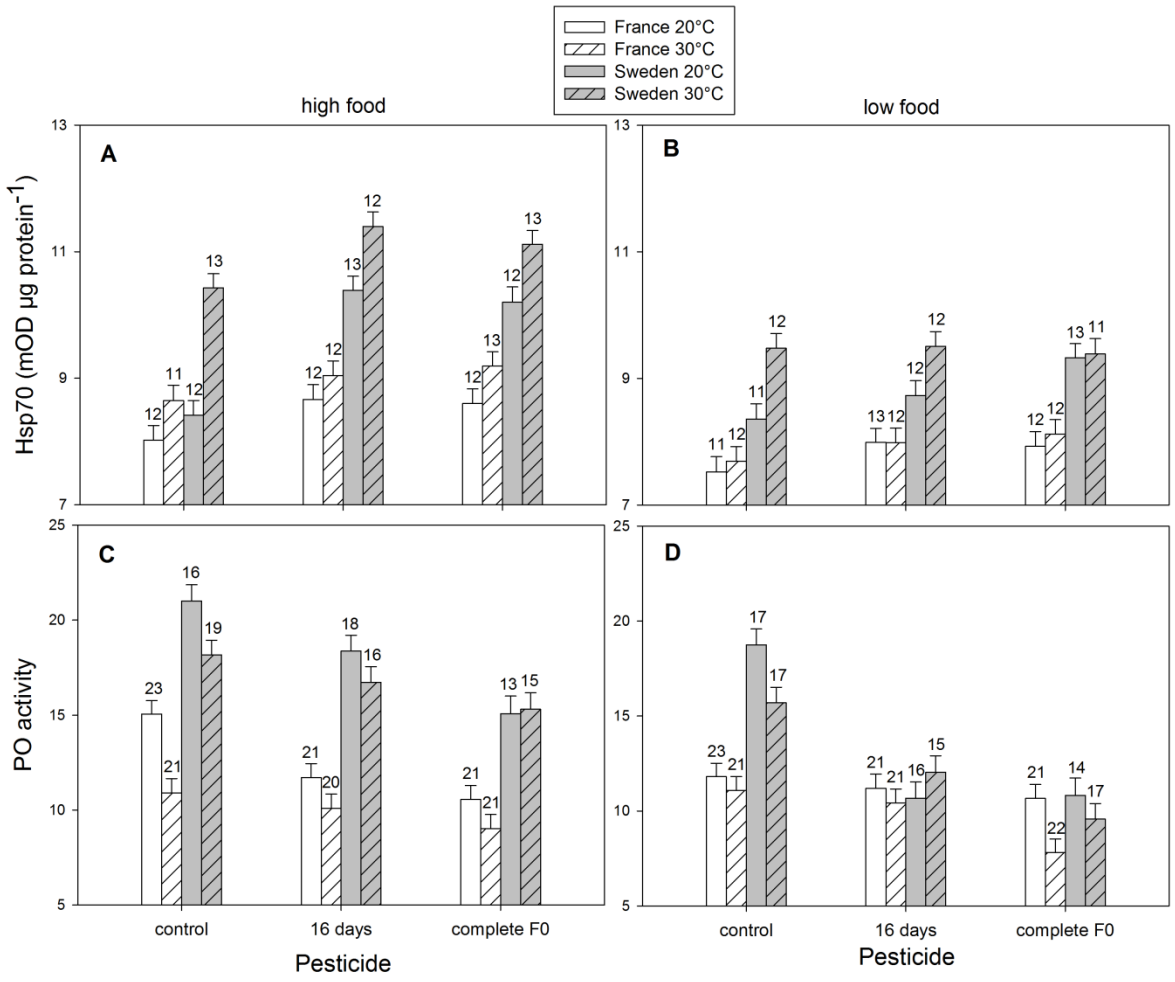
704 **Fig. 2**




705

706

707 **Fig. 3**




708

709

710 Appendix 1. Parameter estimates and their standard errors of the ANCOVAs reported in the manuscript.

711

| Response variable | Effect | Sex | Latitude | Food | Pesticide | Heat | Estimate | Standard Error |
|---|---|---|---|---|---|---|---|---|
| Survial | Latitude (L) | | France | | | | 17.434 | 0.6099 |
| | Latitude (L) | | Sweden | | | | 0 | . |
| | Food (F) | | | high | | | -0.3105 | 0.4907 |
| | Food (F) | | | low | | | 0 | . |
| | Pesticide (P) | | | | control | | 0.3821 | 0.5148 |
| | Pesticide (P) | | | | 16 days | | -0.2495 | 0.4940 |
| | Pesticide (P) | | | | complete F0 | | 0 | . |
| | Heat (H) | | | | | 20°C | -0.1715 | 0.4898 |
| | Heat (H) | | | | | 30°C | 0 | . |
| | L *F | | France | high | | | 19.373 | 12.260 |
| | L * F | | France | low | | | 0 | . |
| | L * F | | Sweden | high | | | 0 | . |
| | L * F | | Sweden | low | | | 0 | . |
| | L * P | | France | | control | | 0.08313 | 0.9287 |
| | L * P | | France | | 16 days | | 0.2683 | 0.8403 |
| | L * P | | France | | complete F0 | | 0 | . |
| | L * P | | Sweden | | control | | 0 | . |
| | L * P | | Sweden | | 16 days | | 0 | . |
| | L * P | | Sweden | | complete F0 | | 0 | . |
| | L * H | | France | | | 20°C | 0.6718 | 0.9145 |
| | L * H | | France | | | 30°C | 0 | . |
| | L * H | | Sweden | | | 20°C | 0 | . |
| | L * H | | Sweden | | | 30°C | 0 | . |
| | F * P | | | high | control | | 0.03914 | 0.7073 |
| | F * P | | | high | 16 days | | 0.4429 | 0.7014 |

| | | | | | | |
|---|---|---|---|---|---|---|
| F * P | | high | complete F0 | | 0 | . |
| F * P | | low | control | | 0 | . |
| F * P | | low | 16 days | | 0 | . |
| F * P | | low | complete F0 | | 0 | . |
| F * H | | high | | 20°C | -0.02337 | 0.6941 |
| F * H | | high | | 30°C | 0 | . |
| F * H | | low | | 20°C | 0 | . |
| F * H | | low | | 30°C | 0 | . |
| P * H | | | control | 20°C | -0.3220 | 0.7103 |
| P * H | | | control | 30°C | 0 | . |
| P * H | | | 16 days | 20°C | 0.3724 | 0.6930 |
| P * H | | | 16 days | 30°C | 0 | . |
| P * H | | | complete F0 | 20°C | 0 | . |
| P * H | | | complete F0 | 30°C | 0 | . |
| L * F * P | France | high | control | | -21.658 | 15.366 |
| L * F * P | France | high | 16 days | | -11.808 | 15.863 |
| L * F * P | France | high | complete F0 | | 0 | . |
| L * F * P | France | low | control | | 0 | . |
| L * F * P | France | low | 16 days | | 0 | . |
| L * F * P | France | low | complete F0 | | 0 | . |
| L * F * P | Sweden | high | control | | 0 | . |
| L * F * P | Sweden | high | 16 days | | 0 | . |
| L * F * P | Sweden | high | complete F0 | | 0 | . |
| L * F * P | Sweden | low | control | | 0 | . |
| L * F * P | Sweden | low | 16 days | | 0 | . |
| L * F * P | Sweden | low | complete F0 | | 0 | . |
| L * F * H | France | high | | 20°C | -18.882 | 15.461 |
| L * F * H | France | high | | 30°C | 0 | . |
| L * F * H | France | low | | 20°C | 0 | . |
| L * F * H | France | low | | 30°C | 0 | . |

| | | | | | | |
|---|---|---|---|---|---|---|
| L * F * H | Sweden | high | | 20°C | 0 | . |
| L * F * H | Sweden | high | | 30°C | 0 | . |
| L * F * H | Sweden | low | | 20°C | 0 | . |
| L * F * H | Sweden | low | | 30°C | 0 | . |
| L * P * H | France | | control | 20°C | 0.9832 | 15.803 |
| L * P * H | France | | control | 30°C | 0 | . |
| L * P * H | France | | 16 days | 20°C | -0.7060 | 12.609 |
| L * P * H | France | | 16 days | 30°C | 0 | . |
| L * P * H | France | | complete F0 | 20°C | 0 | . |
| L * P * H | France | | complete F0 | 30°C | 0 | . |
| L * P * H | Sweden | | control | 20°C | 0 | . |
| L * P * H | Sweden | | control | 30°C | 0 | . |
| L * P * H | Sweden | | 16 days | 20°C | 0 | . |
| L * P * H | Sweden | | 16 days | 30°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 20°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 30°C | 0 | . |
| F * P * H | | high | control | 20°C | 0.6902 | 0.9942 |
| F * P * H | | high | control | 30°C | 0 | . |
| F * P * H | | high | 16 days | 20°C | 0.6226 | 0.9966 |
| F * P * H | | high | 16 days | 30°C | 0 | . |
| F * P * H | | high | complete F0 | 20°C | 0 | . |
| F * P * H | | high | complete F0 | 30°C | 0 | . |
| F * P * H | | low | control | 20°C | 0 | . |
| F * P * H | | low | control | 30°C | 0 | . |
| F * P * H | | low | 16 days | 20°C | 0 | . |
| F * P * H | | low | 16 days | 30°C | 0 | . |
| F * P * H | | low | complete F0 | 20°C | 0 | . |
| F * P * H | | low | complete F0 | 30°C | 0 | . |
| L * F * P * H | France | high | control | 20°C | 0.6287 | 22.113 |
| L * F * P * H | France | high | control | 30°C | 0 | . |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | L * F * P * H | | France | high | 16 days | 20°C | 11.165 | 21.162 |
| | L * F * P * H | | France | high | 16 days | 30°C | 0 | . |
| | L * F * P * H | | France | high | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | France | high | complete F0 | 30°C | 0 | . |
| | L * F * P * H | | France | low | control | 20°C | 0 | . |
| | L * F * P * H | | France | low | control | 30°C | 0 | . |
| | L * F * P * H | | France | low | 16 days | 20°C | 0 | . |
| | L * F * P * H | | France | low | 16 days | 30°C | 0 | . |
| | L * F * P * H | | France | low | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | France | low | complete F0 | 30°C | 0 | . |
| | L * F * P * H | | Sweden | high | control | 20°C | 0 | . |
| | L * F * P * H | | Sweden | high | control | 30°C | 0 | . |
| | L * F * P * H | | Sweden | high | 16 days | 20°C | 0 | . |
| | L * F * P * H | | Sweden | high | 16 days | 30°C | 0 | . |
| | L * F * P * H | | Sweden | high | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | Sweden | high | complete F0 | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | control | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | control | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | 16 days | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | 16 days | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | complete F0 | 30°C | 0 | . |
| Flying ability | Sex | female | | | | | 0.6018 | 0.2315 |
| | Sex | male | | | | | 0 | . |
| | Latitude (L) | | France | | | | -2.1201 | 1.1015 |
| | Latitude (L) | | Sweden | | | | 0 | . |
| | Food (F) | | | high | | | -0.1607 | 1.4662 |
| | Food (F) | | | low | | | 0 | . |
| | Pesticide (P) | | | | control | | -2.5567 | 1.1413 |

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|---|---|---|---|---|---|---|
| Pesticide (P) | | | 16 days | | -3.0253 | 1.1703 |
| Pesticide (P) | | | complete F0 | | 0 | . |
| Heat (H) | | | | 20°C | -0.8390 | 1.2841 |
| Heat (H) | | | | 30°C | 0 | . |
| L * F | France | high | | | 0.5544 | . |
| L * F | France | low | | | 0 | . |
| L * F | Sweden | high | | | 0 | . |
| L * F | Sweden | low | | | 0 | . |
| L * P | France | | control | | 2.6063 | 1.2623 |
| L * P | France | | 16 days | | 2.7397 | 1.2801 |
| L * P | France | | complete F0 | | 0 | . |
| L * P | Sweden | | control | | 0 | . |
| L * P | Sweden | | 16 days | | 0 | . |
| L * P | Sweden | | complete F0 | | 0 | . |
| L * H | France | | | 20°C | 2.4270 | 1.4699 |
| L * H | France | | | 30°C | 0 | . |
| L * H | Sweden | | | 20°C | 0 | . |
| L * H | Sweden | | | 30°C | 0 | . |
| F * P | | high | control | | -0.5464 | 1.6097 |
| F * P | | high | 16 days | | 1.9350 | 1.6923 |
| F * P | | high | complete F0 | | 0 | . |
| F * P | | low | control | | 0 | . |
| F * P | | low | 16 days | | 0 | . |
| F * P | | low | complete F0 | | 0 | . |
| F * H | | high | | 20°C | -1.1325 | 1.7449 |
| F * H | | high | | 30°C | 0 | . |
| F * H | | low | | 20°C | 0 | . |
| F * H | | low | | 30°C | 0 | . |
| P * H | | | control | 20°C | 3.4063 | 1.7169 |
| P * H | | | control | 30°C | 0 | . |

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|---|---|---|---|---|---|---|
| P * H | | | 16 days | 20°C | 3.1795 | 1.5874 |
| P * H | | | 16 days | 30°C | 0 | . |
| P * H | | | complete F0 | 20°C | 0 | . |
| P * H | | | complete F0 | 30°C | 0 | . |
| L * F * P | France | high | control | | 0.3297 | 1.7939 |
| L * F * P | France | high | 16 days | | -1.9363 | 1.8601 |
| L * F * P | France | high | complete F0 | | 0 | . |
| L * F * P | France | low | control | | 0 | . |
| L * F * P | France | low | 16 days | | 0 | . |
| L * F * P | France | low | complete F0 | | 0 | . |
| L * F * P | Sweden | high | control | | 0 | . |
| L * F * P | Sweden | high | 16 days | | 0 | . |
| L * F * P | Sweden | high | complete F0 | | 0 | . |
| L * F * P | Sweden | low | control | | 0 | . |
| L * F * P | Sweden | low | 16 days | | 0 | . |
| L * F * P | Sweden | low | complete F0 | | 0 | . |
| L * F * H | France | high | | 20°C | 1.2245 | 2.0653 |
| L * F * H | France | high | | 30°C | 0 | . |
| L * F * H | France | low | | 20°C | 0 | . |
| L * F * H | France | low | | 30°C | 0 | . |
| L * F * H | Sweden | high | | 20°C | 0 | . |
| L * F * H | Sweden | high | | 30°C | 0 | . |
| L * F * H | Sweden | low | | 20°C | 0 | . |
| L * F * H | Sweden | low | | 30°C | 0 | . |
| L * P * H | France | | control | 20°C | -3.6558 | 1.9738 |
| L * P * H | France | | control | 30°C | 0 | . |
| L * P * H | France | | 16 days | 20°C | -2.8637 | 1.8786 |
| L * P * H | France | | 16 days | 30°C | 0 | . |
| L * P * H | France | | complete F0 | 20°C | 0 | . |
| L * P * H | France | | complete F0 | 30°C | 0 | . |

| | | | | | | |
|---|---|---|---|---|---|---|
| L * P * H | Sweden | | control | 20°C | 0 | . |
| L * P * H | Sweden | | control | 30°C | 0 | . |
| L * P * H | Sweden | | 16 days | 20°C | 0 | . |
| L * P * H | Sweden | | 16 days | 30°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 20°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 30°C | 0 | . |
| F * P * H | | high | control | 20°C | 2.0441 | 2.3688 |
| F * P * H | | high | control | 30°C | 0 | . |
| F * P * H | | high | 16 days | 20°C | -0.9099 | 2.1739 |
| F * P * H | | high | 16 days | 30°C | 0 | . |
| F * P * H | | high | complete F0 | 20°C | 0 | . |
| F * P * H | | high | complete F0 | 30°C | 0 | . |
| F * P * H | | low | control | 20°C | 0 | . |
| F * P * H | | low | control | 30°C | 0 | . |
| F * P * H | | low | 16 days | 20°C | 0 | . |
| F * P * H | | low | 16 days | 30°C | 0 | . |
| F * P * H | | low | complete F0 | 20°C | 0 | . |
| F * P * H | | low | complete F0 | 30°C | 0 | . |
| L * F * P * H | France | high | control | 20°C | -1.9994 | 2.7947 |
| L * F * P * H | France | high | control | 30°C | 0 | . |
| L * F * P * H | France | high | 16 days | 20°C | 0.8865 | 2.6710 |
| L * F * P * H | France | high | 16 days | 30°C | 0 | . |
| .L * F * P * H | France | high | complete F0 | 20°C | 0 | . |
| L * F * P * H | France | high | complete F0 | 30°C | 0 | . |
| L * F * P * H | France | low | control | 20°C | 0 | . |
| L * F * P * H | France | low | control | 30°C | 0 | . |
| L * F * P * H | France | low | 16 days | 20°C | 0 | . |
| L * F * P * H | France | low | 16 days | 30°C | 0 | . |
| L * F * P * H | France | low | complete F0 | 20°C | 0 | . |
| L * F * P * H | France | low | complete F0 | 30°C | 0 | . |

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|---|---|---|---|---|---|---|---|---|
| | L * F * P * H | | Sweden | high | control | 20°C | 0 | . |
| | L * F * P * H | | Sweden | high | control | 30°C | 0 | . |
| | L * F * P * H | | Sweden | high | 16 days | 20°C | 0 | . |
| | L * F * P * H | | Sweden | high | 16 days | 30°C | 0 | . |
| | L * F * P * H | | Sweden | high | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | Sweden | high | complete F0 | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | control | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | control | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | 16 days | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | 16 days | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | complete F0 | 30°C | 0 | . |
| Development time | Sex | female | | | | | 0.7022 | 0.8827 |
| | Sex | male | | | | | 0 | . |
| | Latitude (L) | | France | | | | -39.8475 | 2.2719 |
| | Latitude (L) | | Sweden | | | | 0 | . |
| | Food (F) | | | high | | | 4.2302 | 2.6304 |
| | Food (F) | | | low | | | 0 | . |
| | Pesticide (P) | | | | control | | 7.1435 | 2.5745 |
| | Pesticide (P) | | | | 16 days | | 4.4214 | 2.6512 |
| | Pesticide (P) | | | | complete F0 | | 0 | . |
| | L * F | | France | high | | | -7.8675 | 3.22865 |
| | L * F | | France | low | | | 0 | . |
| | L * F | | Sweden | high | | | 0 | . |
| | L * F | | Sweden | low | | | 0 | . |
| | L * P | | France | | control | | -6.4352 | 3.1724 |
| | L * P | | France | | 16 days | | -4.4343 | 3.2337 |
| | L * P | | France | | complete F0 | | 0 | . |
| | L * P | | Sweden | | control | | 0 | . |

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|---|---|---|---|---|---|---|---|
| | L * P | | Sweden | | 16 days | 0 | . |
| | L * P | | Sweden | | complete F0 | 0 | . |
| | F * P | | | high | control | -14.1857 | 3.6077 |
| | F * P | | | high | 16 days | -10.0882 | 3.6722 |
| | F * P | | | high | complete F0 | 0 | . |
| | F * P | | | low | control | 0 | . |
| | F * P | | | low | 16 days | 0 | . |
| | F * P | | | low | complete F0 | 0 | . |
| | L * F * P | | France | high | control | 12.6929 | 4.4734 |
| | L * F * P | | France | high | 16 days | 9.5663 | 4.5321 |
| | L * F * P | | France | high | complete F0 | 0 | . |
| | L * F * P | | France | low | control | 0 | . |
| | L * F * P | | France | low | 16 days | 0 | . |
| | L * F * P | | France | low | complete F0 | 0 | . |
| | L * F * P | | Sweden | high | control | 0 | . |
| | L * F * P | | Sweden | high | 16 days | 0 | . |
| | L * F * P | | Sweden | high | complete F0 | 0 | . |
| | L * F * P | | Sweden | low | control | 0 | . |
| | L * F * P | | Sweden | low | 16 days | 0 | . |
| | L * F * P | | Sweden | low | complete F0 | 0 | . |
| Dry mass | Sex | female | | | | 0.1664 | 0.02774 |
| | Sex | male | | | | 0 | . |
| | Latitude (L) | | France | | | -0.6463 | 0.1643 |
| | Latitude (L) | | Sweden | | | 0 | . |
| | Food (F) | | | high | | 0.2687 | 0.1019 |
| | Food (F) | | | low | | 0 | . |
| | Pesticide (P) | | | | control | -0.01375 | 0.09780 |
| | Pesticide (P) | | | | 16 days | 0.04344 | 0.1010 |
| | Pesticide (P) | | | | complete F0 | 0 | . |

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|---|---|---|---|---|---|---|
| Heat (H) | | | | 20°C | 0.02070 | 0.1039 |
| Heat (H) | | | | 30°C | 0 | . |
| L * F | France | high | | | 0.1283 | 0.1346 |
| L * F | France | low | | | 0 | . |
| L * F | Sweden | high | | | 0 | . |
| L * F | Sweden | low | | | 0 | . |
| L * P | France | | control | | 0.03015 | 0.1309 |
| L * P | France | | 16 days | | 0.01611 | 0.1332 |
| L * P | France | | complete F0 | | 0 | . |
| L * P | Sweden | | control | | 0 | . |
| L * P | Sweden | | 16 days | | 0 | . |
| L * P | Sweden | | complete F0 | | 0 | . |
| L * H | France | | | 20°C | 0.04574 | 0.1355 |
| L * H | France | | | 30°C | 0 | . |
| L * H | Sweden | | | 20°C | 0 | . |
| L * H | Sweden | | | 30°C | 0 | . |
| F * P | | high | control | | 0.01134 | 0.1389 |
| F * P | | high | 16 days | | -0.2112 | 0.1451 |
| F * P | | high | complete F0 | | 0 | . |
| F * P | | low | control | | 0 | . |
| F * P | | low | 16 days | | 0 | . |
| F * P | | low | complete F0 | | 0 | . |
| F * H | | high | | 20°C | -0.1215 | 0.1504 |
| F * H | | high | | 30°C | 0 | . |
| F * H | | low | | 20°C | 0 | . |
| F * H | | low | | 30°C | 0 | . |
| P * H | | | control | 20°C | 0.1058 | 0.1427 |
| P * H | | | control | 30°C | 0 | . |
| P * H | | | 16 days | 20°C | 0.02412 | 0.1457 |
| P * H | | | 16 days | 30°C | 0 | . |

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|---|---|---|---|---|---|---|
| P * H | | | complete F0 | 20°C | 0 | . |
| P * H | | | complete F0 | 30°C | 0 | . |
| L * F * P | France | high | control | | 0.1767 | 0.1870 |
| L * F * P | France | high | 16 days | | 0.1828 | 0.1916 |
| L * F * P | France | high | complete F0 | | 0 | . |
| L * F * P | France | low | control | | 0 | . |
| L * F * P | France | low | 16 days | | 0 | . |
| L * F * P | France | low | complete F0 | | 0 | . |
| L * F * P | Sweden | high | control | | 0 | . |
| L * F * P | Sweden | high | 16 days | | 0 | . |
| L * F * P | Sweden | high | complete F0 | | 0 | . |
| L * F * P | Sweden | low | control | | 0 | . |
| L * F * P | Sweden | low | 16 days | | 0 | . |
| L * F * P | Sweden | low | complete F0 | | 0 | . |
| L * F * H | France | high | | 20°C | 0.1629 | 0.1951 |
| L * F * H | France | high | | 30°C | 0 | . |
| L * F * H | France | low | | 20°C | 0 | . |
| L * F * H | France | low | | 30°C | 0 | . |
| L * F * H | Sweden | high | | 20°C | 0 | . |
| L * F * H | Sweden | high | | 30°C | 0 | . |
| L * F * H | Sweden | low | | 20°C | 0 | . |
| L * F * H | Sweden | low | | 30°C | 0 | . |
| L * P * H | France | | control | 20°C | 0.01017 | 0.1881 |
| L * P * H | France | | control | 30°C | 0 | . |
| L * P * H | France | | 16 days | 20°C | -0.04670 | 0.1911 |
| L * P * H | France | | 16 days | 30°C | 0 | . |
| L * P * H | France | | complete F0 | 20°C | 0 | . |
| L * P * H | France | | complete F0 | 30°C | 0 | . |
| L * P * H | Sweden | | control | 20°C | 0 | . |
| L * P * H | Sweden | | control | 30°C | 0 | . |

| | | | | | | |
|---|---|---|---|---|---|---|
| L * P * H | Sweden | | 16 days | 20°C | 0 | . |
| L * P * H | Sweden | | 16 days | 30°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 20°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 30°C | 0 | . |
| F * P * H | | high | control | 20°C | -0.00037 | 0.2031 |
| F * P * H | | high | control | 30°C | 0 | . |
| F * P * H | | high | 16 days | 20°C | 0.2163 | 0.2077 |
| F * P * H | | high | 16 days | 30°C | 0 | . |
| F * P * H | | high | complete F0 | 20°C | 0 | . |
| F * P * H | | high | complete F0 | 30°C | 0 | . |
| F * P * H | | low | control | 20°C | 0 | . |
| F * P * H | | low | control | 30°C | 0 | . |
| F * P * H | | low | 16 days | 20°C | 0 | . |
| F * P * H | | low | 16 days | 30°C | 0 | . |
| F * P * H | | low | complete F0 | 20°C | 0 | . |
| F * P * H | | low | complete F0 | 30°C | 0 | . |
| L * F * P * H | France | high | control | 20°C | -0.3000 | 0.2681 |
| L * F * P * H | France | high | control | 30°C | 0 | . |
| L * F * P * H | France | high | 16 days | 20°C | -0.2729 | 0.2725 |
| L * F * P * H | France | high | 16 days | 30°C | 0 | . |
| L * F * P * H | France | high | complete F0 | 20°C | 0 | . |
| L * F * P * H | France | high | complete F0 | 30°C | 0 | . |
| L * F * P * H | France | low | control | 20°C | 0 | . |
| L * F * P * H | France | low | control | 30°C | 0 | . |
| L * F * P * H | France | low | 16 days | 20°C | 0 | . |
| L * F * P * H | France | low | 16 days | 30°C | 0 | . |
| L * F * P * H | France | low | complete F0 | 20°C | 0 | . |
| L * F * P * H | France | low | complete F0 | 30°C | 0 | . |
| L * F * P * H | Sweden | high | control | 20°C | 0 | . |
| L * F * P * H | Sweden | high | control | 30°C | 0 | . |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | L * F * P * H | | Sweden | high | 16 days | 20°C | 0 | . |
| | L * F * P * H | | Sweden | high | 16 days | 30°C | 0 | . |
| | L * F * P * H | | Sweden | high | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | Sweden | high | complete F0 | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | control | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | control | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | 16 days | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | 16 days | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | complete F0 | 30°C | 0 | . |
| Water content | Sex | female | | | | | 0.1763 | 0.06624 |
| | Sex | male | | | | | 0 | . |
| | Latitude (L) | | France | | | | 0.7253 | 0.2508 |
| | Latitude (L) | | Sweden | | | | 0 | . |
| | Food (F) | | | high | | | 0.1725 | 0.2425 |
| | Food (F) | | | low | | | 0 | . |
| | Pesticide (P) | | | | control | | -0.1158 | 0.2332 |
| | Pesticide (P) | | | | 16 days | | -0.6174 | 0.2408 |
| | Pesticide (P) | | | | complete F0 | | 0 | . |
| | Heat (H) | | | | | 20°C | 0.3922 | 0.2474 |
| | Heat (H) | | | | | 30°C | 0 | . |
| | L *F | | France | high | | | -0.5717 | 0.3207 |
| | L * F | | France | low | | | 0 | . |
| | L * F | | Sweden | high | | | 0 | . |
| | L * F | | Sweden | low | | | 0 | . |
| | L * P | | France | | control | | 0.04289 | 0.3123 |
| | L * P | | France | | 16 days | | 0.5954 | 0.3178 |
| | L * P | | France | | complete F0 | | 0 | . |
| | L * P | | Sweden | | control | | 0 | . |

| | | | | | | |
|---|---|---|---|---|---|---|
| L * P | Sweden | | 16 days | | 0 | . |
| L * P | Sweden | | complete F0 | | 0 | . |
| L * H | France | | | 20°C | -0.05808 | 0.3228 |
| L * H | France | | | 30°C | 0 | . |
| L * H | Sweden | | | 20°C | 0 | . |
| L * H | Sweden | | | 30°C | 0 | . |
| F * P | | high | control | | -0.2526 | 0.3311 |
| F * P | | high | 16 days | | 0.3095 | 0.3453 |
| F * P | | high | complete F0 | | 0 | . |
| F * P | | low | control | | 0 | . |
| F * P | | low | 16 days | | 0 | . |
| F * P | | low | complete F0 | | 0 | . |
| F * H | | high | | 20°C | -0.2641 | 0.3582 |
| F * H | | high | | 30°C | 0 | . |
| F * H | | low | | 20°C | 0 | . |
| F * H | | low | | 30°C | 0 | . |
| P * H | | | control | 20°C | 0.03193 | 0.3422 |
| P * H | | | control | 30°C | 0 | . |
| P * H | | | 16 days | 20°C | 0.7001 | 0.3503 |
| P * H | | | 16 days | 30°C | 0 | . |
| P * H | | | complete F0 | 20°C | 0 | . |
| P * H | | | complete F0 | 30°C | 0 | . |
| L * F * P | France | high | control | | -0.00554 | 0.4460 |
| L * F * P | France | high | 16 days | | 0 | . |
| L * F * P | France | high | complete F0 | | -0.4651 | 0.4566 |
| L * F * P | France | low | control | | 0 | . |
| L * F * P | France | low | 16 days | | 0 | . |
| L * F * P | France | low | complete F0 | | 0 | . |
| L * F * P | Sweden | high | control | | 0 | . |
| L * F * P | Sweden | high | 16 days | | 0 | . |

| | | | | | | |
|---|---|---|---|---|---|---|
| L * F * P | Sweden | high | complete F0 | | 0 | . |
| L * F * P | Sweden | low | control | | 0 | . |
| L * F * P | Sweden | low | 16 days | | 0 | . |
| L * F * P | Sweden | low | complete F0 | | 0 | . |
| L * F * H | France | high | | 20°C | 0.2544 | 0.4649 |
| L * F * H | France | high | | 30°C | 0 | . |
| L * F * H | France | low | | 20°C | 0 | . |
| L * F * H | France | low | | 30°C | 0 | . |
| L * F * H | Sweden | high | | 20°C | 0 | . |
| L * F * H | Sweden | high | | 30°C | 0 | . |
| L * F * H | Sweden | low | | 20°C | 0 | . |
| L * F * H | Sweden | low | | 30°C | 0 | . |
| L * P * H | France | | control | 20°C | -0.1374 | 0.4502 |
| L * P * H | France | | control | 30°C | 0 | . |
| L * P * H | France | | 16 days | 20°C | -0.8883 | 0.4579 |
| L * P * H | France | | 16 days | 30°C | 0 | . |
| L * P * H | France | | complete F0 | 20°C | 0 | . |
| L * P * H | France | | complete F0 | 30°C | 0 | . |
| L * P * H | Sweden | | control | 20°C | 0 | . |
| L * P * H | Sweden | | control | 30°C | 0 | . |
| L * P * H | Sweden | | 16 days | 20°C | 0 | . |
| L * P * H | Sweden | | 16 days | 30°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 20°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 30°C | 0 | . |
| F * P * H | | high | control | 20°C | 0.09846 | 0.4859 |
| F * P * H | | high | control | 30°C | 0 | . |
| F * P * H | | high | 16 days | 20°C | -0.4577 | 0.4987 |
| F * P * H | | high | 16 days | 30°C | 0 | . |
| F * P * H | | high | complete F0 | 20°C | 0 | . |
| F * P * H | | high | complete F0 | 30°C | 0 | . |

| | | | | | | |
|---|---|---|---|---|---|---|
| F * P * H | | low | control | 20°C | 0 | . |
| F * P * H | | low | control | 30°C | 0 | . |
| F * P * H | | low | 16 days | 20°C | 0 | . |
| F * P * H | | low | 16 days | 30°C | 0 | . |
| F * P * H | | low | complete F0 | 20°C | 0 | . |
| F * P * H | | low | complete F0 | 30°C | 0 | . |
| L * F * P * H | France | high | control | 20°C | 0.3139 | 0.6407 |
| L * F * P * H | France | high | control | 30°C | 0 | . |
| L * F * P * H | France | high | 16 days | 20°C | 0.7531 | 0.6524 |
| L * F * P * H | France | high | 16 days | 30°C | 0 | . |
| L * F * P * H | France | high | complete F0 | 20°C | 0 | . |
| L * F * P * H | France | high | complete F0 | 30°C | 0 | . |
| L * F * P * H | France | low | control | 20°C | 0 | . |
| L * F * P * H | France | low | control | 30°C | 0 | . |
| L * F * P * H | France | low | 16 days | 20°C | 0 | . |
| L * F * P * H | France | low | 16 days | 30°C | 0 | . |
| L * F * P * H | France | low | complete F0 | 20°C | 0 | . |
| L * F * P * H | France | low | complete F0 | 30°C | 0 | . |
| L * F * P * H | Sweden | high | control | 20°C | 0 | . |
| L * F * P * H | Sweden | high | control | 30°C | 0 | . |
| L * F * P * H | Sweden | high | 16 days | 20°C | 0 | . |
| L * F * P * H | Sweden | high | 16 days | 30°C | 0 | . |
| L * F * P * H | Sweden | high | complete F0 | 20°C | 0 | . |
| L * F * P * H | Sweden | high | complete F0 | 30°C | 0 | . |
| L * F * P * H | Sweden | low | control | 20°C | 0 | . |
| L * F * P * H | Sweden | low | control | 30°C | 0 | . |
| L * F * P * H | Sweden | low | 16 days | 20°C | 0 | . |
| L * F * P * H | Sweden | low | 16 days | 30°C | 0 | . |
| L * F * P * H | Sweden | low | complete F0 | 20°C | 0 | . |
| L * F * P * H | Sweden | low | complete F0 | 30°C | 0 | . |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Fat content | Sex | female | | | | | -0.1374 | 0.1118 |
| | Sex | male | | | | | 0 | . |
| | Latitude (L) | | France | | | | -1.8754 | 0.3939 |
| | Latitude (L) | | Sweden | | | | 0 | . |
| | Food (F) | | | high | | | 1.0003 | 0.4471 |
| | Food (F) | | | low | | | 0 | . |
| | Pesticide (P) | | | | control | | 0.1377 | 0.4257 |
| | Pesticide (P) | | | | 16 days | | -0.7559 | 0.4180 |
| | Pesticide (P) | | | | complete F0 | | 0 | . |
| | Heat (H) | | | | | 20°C | 0.09057 | 0.4195 |
| | Heat (H) | | | | | 30°C | 0 | . |
| | L *F | | France | high | | | 0.5809 | 0.5657 |
| | L * F | | France | low | | | 0 | . |
| | L * F | | Sweden | high | | | 0 | . |
| | L * F | | Sweden | low | | | 0 | . |
| | L * P | | France | | control | | 0.1539 | 0.5472 |
| | L * P | | France | | 16 days | | 0.8839 | 0.5403 |
| | L * P | | France | | complete F0 | | 0 | . |
| | L * P | | Sweden | | control | | 0 | . |
| | L * P | | Sweden | | 16 days | | 0 | . |
| | L * P | | Sweden | | complete F0 | | 0 | . |
| | L * H | | France | | | 20°C | 1.0166 | 0.5416 |
| | L * H | | France | | | 30°C | 0 | . |
| | L * H | | Sweden | | | 20°C | 0 | . |
| | L * H | | Sweden | | | 30°C | 0 | . |
| | F * P | | | high | control | | -0.17194 | 0.4080 |
| | F * P | | | high | 16 days | | 0.5620 | 0.4136 |
| | F * P | | | high | complete F0 | | 0 | . |
| | F * P | | | low | control | | 0 | . |

| | | | | | | |
|---|---|---|---|---|---|---|
| F * P | | low | 16 days | | 0 | . |
| F * P | | low | complete F0 | | 0 | . |
| F * H | | high | | 20°C | -0.2412 | 0.6475 |
| F * H | | high | | 30°C | 0 | . |
| F * H | | low | | 20°C | 0 | . |
| F * H | | low | | 30°C | 0 | . |
| P * H | | | control | 20°C | 0.2031 | 0.4935 |
| P * H | | | control | 30°C | 0 | . |
| P * H | | | 16 days | 20°C | 0.9843 | 0.4935 |
| P * H | | | 16 days | 30°C | 0 | . |
| P * H | | | complete F0 | 20°C | 0 | . |
| P * H | | | complete F0 | 30°C | 0 | . |
| L * F * P | France | high | control | | 0.2943 | 0.7831 |
| L * F * P | France | high | 16 days | | -0.6110 | 0.7874 |
| L * F * P | France | high | complete F0 | | 0 | . |
| L * F * P | France | low | control | | 0 | . |
| L * F * P | France | low | 16 days | | 0 | . |
| L * F * P | France | low | complete F0 | | 0 | . |
| L * F * P | Sweden | high | control | | 0 | . |
| L * F * P | Sweden | high | 16 days | | 0 | . |
| L * F * P | Sweden | high | complete F0 | | 0 | . |
| L * F * P | Sweden | low | control | | 0 | . |
| L * F * P | Sweden | low | 16 days | | 0 | . |
| L * F * P | Sweden | low | complete F0 | | 0 | . |
| L * F * H | France | high | | 20°C | -0.1882 | 0.8114 |
| L * F * H | France | high | | 30°C | 0 | . |
| L * F * H | France | low | | 20°C | 0 | . |
| L * F * H | France | low | | 30°C | 0 | . |
| L * F * H | Sweden | high | | 20°C | 0 | . |
| L * F * H | Sweden | high | | 30°C | 0 | . |

| | | | | | | |
|---|---|---|---|---|---|---|
| L * F * H | Sweden | low | | 20°C | 0 | . |
| L * F * H | Sweden | low | | 30°C | 0 | . |
| L * P * H | France | | control | 20°C | -0.5483 | 0.7656 |
| L * P * H | France | | control | 30°C | 0 | . |
| L * P * H | France | | 16 days | 20°C | -1.1392 | 0.7670 |
| L * P * H | France | | 16 days | 30°C | 0 | . |
| L * P * H | France | | complete F0 | 20°C | 0 | . |
| L * P * H | France | | complete F0 | 30°C | 0 | . |
| L * P * H | Sweden | | control | 20°C | 0 | . |
| L * P * H | Sweden | | control | 30°C | 0 | . |
| L * P * H | Sweden | | 16 days | 20°C | 0 | . |
| L * P * H | Sweden | | 16 days | 30°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 20°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 30°C | 0 | . |
| F * P * H | | high | control | 20°C | 0.2630 | 0.8625 |
| F * P * H | | high | control | 30°C | 0 | . |
| F * P * H | | high | 16 days | 20°C | 0.08102 | 0.8686 |
| F * P * H | | high | 16 days | 30°C | 0 | . |
| F * P * H | | high | complete F0 | 20°C | 0 | . |
| F * P * H | | high | complete F0 | 30°C | 0 | . |
| F * P * H | | low | control | 20°C | 0 | . |
| F * P * H | | low | control | 30°C | 0 | . |
| F * P * H | | low | 16 days | 20°C | 0 | . |
| F * P * H | | low | 16 days | 30°C | 0 | . |
| F * P * H | | low | complete F0 | 20°C | 0 | . |
| F * P * H | | low | complete F0 | 30°C | 0 | . |
| L * F * P * H | France | high | control | 20°C | -0.08763 | 1.1049 |
| L * F * P * H | France | high | control | 30°C | 0 | . |
| L * F * P * H | France | high | 16 days | 20°C | -0.1268 | 1.1122 |
| L * F * P * H | France | high | 16 days | 30°C | 0 | . |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | L * F * P * H | | France | high | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | France | high | complete F0 | 30°C | 0 | . |
| | L * F * P * H | | France | low | control | 20°C | 0 | . |
| | L * F * P * H | | France | low | control | 30°C | 0 | . |
| | L * F * P * H | | France | low | 16 days | 20°C | 0 | . |
| | L * F * P * H | | France | low | 16 days | 30°C | 0 | . |
| | L * F * P * H | | France | low | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | France | low | complete F0 | 30°C | 0 | . |
| | L * F * P * H | | Sweden | high | control | 20°C | 0 | . |
| | L * F * P * H | | Sweden | high | control | 30°C | 0 | . |
| | L * F * P * H | | Sweden | high | 16 days | 20°C | 0 | . |
| | L * F * P * H | | Sweden | high | 16 days | 30°C | 0 | . |
| | L * F * P * H | | Sweden | high | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | Sweden | high | complete F0 | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | control | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | control | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | 16 days | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | 16 days | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | complete F0 | 30°C | 0 | . |
| Hsp70 levels | Sex | female | | | | | -0.1563 | 0.0963 |
| | Sex | male | | | | | 0 | . |
| | Latitude (L) | | France | | | | -1.2342 | 0.3346 |
| | Latitude (L) | | Sweden | | | | 0 | . |
| | Food (F) | | | high | | | 1.7707 | 0.3286 |
| | Food (F) | | | low | | | 0 | . |
| | Pesticide (P) | | | | control | | 0.1167 | 0.3346 |
| | Pesticide (P) | | | | 16 days | | 0.1298 | 0.3344 |
| | Pesticide (P) | | | | complete F0 | | 0 | . |

| | | | | | | |
|---|---|---|---|---|---|---|
| Heat (H) | | | | 20°C | -0.03578 | 0.3283 |
| Heat (H) | | | | 30°C | 0 | . |
| L * F | France | high | | | -0.6891 | 0.4590 |
| L * F | France | low | | | 0 | . |
| L * F | Sweden | high | | | 0 | . |
| L * F | Sweden | low | | | 0 | . |
| L * P | France | | control | | -0.5536 | 0.4679 |
| L * P | France | | 16 days | | -0.2715 | 0.4677 |
| L * P | France | | complete F0 | | 0 | . |
| L * P | Sweden | | control | | 0 | . |
| L * P | Sweden | | 16 days | | 0 | . |
| L * P | Sweden | | complete F0 | | 0 | . |
| L * H | France | | | 20°C | -0.1543 | 0.4634 |
| L * H | France | | | 30°C | 0 | . |
| L * H | Sweden | | | 20°C | 0 | . |
| L * H | Sweden | | | 30°C | 0 | . |
| F * P | | high | control | | -0.7994 | 0.4589 |
| F * P | | high | 16 days | | 0.1423 | 0.4687 |
| F * P | | high | complete F0 | | 0 | . |
| F * P | | low | control | | 0 | . |
| F * P | | low | 16 days | | 0 | . |
| F * P | | low | complete F0 | | 0 | . |
| F * H | | high | | 20°C | -0.9188 | 0.4649 |
| F * H | | high | | 30°C | 0 | . |
| F * H | | low | | 20°C | 0 | . |
| F * H | | low | | 30°C | 0 | . |
| P * H | | | control | 20°C | -1.0935 | 0.4687 |
| P * H | | | control | 30°C | 0 | . |
| P * H | | | 16 days | 20°C | -0.7499 | 0.4636 |
| P * H | | | 16 days | 30°C | 0 | . |

| | | | | | | |
|---|---|---|---|---|---|---|
| P * H | | | complete F0 | 20°C | 0 | . |
| P * H | | | complete F0 | 30°C | 0 | . |
| L * F * P | France | high | control | | 0.6761 | 0.6521 |
| L * F * P | France | high | 16 days | | -0.1333 | 0.6589 |
| L * F * P | France | high | complete F0 | | 0 | . |
| L * F * P | France | low | control | | 0 | . |
| L * F * P | France | low | 16 days | | 0 | . |
| L * F * P | France | low | complete F0 | | 0 | . |
| L * F * P | Sweden | high | control | | 0 | . |
| L * F * P | Sweden | high | 16 days | | 0 | . |
| L * F * P | Sweden | high | complete F0 | | 0 | . |
| L * F * P | Sweden | low | control | | 0 | . |
| L * F * P | Sweden | low | 16 days | | 0 | . |
| L * F * P | Sweden | low | complete F0 | | 0 | . |
| L * F * H | France | high | | 20°C | 0.4769 | 0.6521 |
| L * F * H | France | high | | 30°C | 0 | . |
| L * F * H | France | low | | 20°C | 0 | . |
| L * F * H | France | low | | 30°C | 0 | . |
| L * F * H | Sweden | high | | 20°C | 0 | . |
| L * F * H | Sweden | high | | 30°C | 0 | . |
| L * F * H | Sweden | low | | 20°C | 0 | . |
| L * F * H | Sweden | low | | 30°C | 0 | . |
| L * P * H | France | | control | 20°C | 1.0826 | 0.6621 |
| L * P * H | France | | control | 30°C | 0 | . |
| L * P * H | France | | 16 days | 20°C | 0.9435 | 0.6516 |
| L * P * H | France | | 16 days | 30°C | 0 | . |
| L * P * H | France | | complete F0 | 20°C | 0 | . |
| L * P * H | France | | complete F0 | 30°C | 0 | . |
| L * P * H | Sweden | | control | 20°C | 0 | . |
| L * P * H | Sweden | | control | 30°C | 0 | . |

| | | | | | | |
|---|---|---|---|---|---|---|
| L * P * H | Sweden | | 16 days | 20°C | 0 | . |
| L * P * H | Sweden | | 16 days | 30°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 20°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 30°C | 0 | . |
| F * P * H | | high | control | 20°C | 0.006498 | 0.6559 |
| F * P * H | | high | control | 30°C | 0 | . |
| F * P * H | | high | 16 days | 20°C | 0.6848 | 0.6567 |
| F * P * H | | high | 16 days | 30°C | 0 | . |
| F * P * H | | high | complete F0 | 20°C | 0 | . |
| F * P * H | | high | complete F0 | 30°C | 0 | . |
| F * P * H | | low | control | 20°C | 0 | . |
| F * P * H | | low | control | 30°C | 0 | . |
| F * P * H | | low | 16 days | 20°C | 0 | . |
| F * P * H | | low | 16 days | 30°C | 0 | . |
| F * P * H | | low | complete F0 | 20°C | 0 | . |
| F * P * H | | low | complete F0 | 30°C | 0 | . |
| L * F * P * H | France | high | control | 20°C | -0.02101 | 0.9245 |
| L * F * P * H | France | high | control | 30°C | 0 | . |
| L * F * P * H | France | high | 16 days | 20°C | -0.6629 | 0.9245 |
| L * F * P * H | France | high | 16 days | 30°C | 0 | . |
| L * F * P * H | France | high | complete F0 | 20°C | 0 | . |
| L * F * P * H | France | high | complete F0 | 30°C | 0 | . |
| L * F * P * H | France | low | control | 20°C | 0 | . |
| L * F * P * H | France | low | control | 30°C | 0 | . |
| L * F * P * H | France | low | 16 days | 20°C | 0 | . |
| L * F * P * H | France | low | 16 days | 30°C | 0 | . |
| L * F * P * H | France | low | complete F0 | 20°C | 0 | . |
| L * F * P * H | France | low | complete F0 | 30°C | 0 | . |
| L * F * P * H | Sweden | high | control | 20°C | 0 | . |
| L * F * P * H | Sweden | high | control | 30°C | 0 | . |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | L * F * P * H | | Sweden | high | 16 days | 20°C | 0 | . |
| | L * F * P * H | | Sweden | high | 16 days | 30°C | 0 | . |
| | L * F * P * H | | Sweden | high | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | Sweden | high | complete F0 | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | control | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | control | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | 16 days | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | 16 days | 30°C | 0 | . |
| | L * F * P * H | | Sweden | low | complete F0 | 20°C | 0 | . |
| | L * F * P * H | | Sweden | low | complete F0 | 30°C | 0 | . |
| PO activity | protein | | | | | | 9.7817 | 5.0519 |
| | mass | | | | | | 0.04709 | 0.07243 |
| | Sex | female | | | | | -0.09805 | 0.3439 |
| | Sex | male | | | | | 0 | . |
| | Latitude (L) | | France | | | | -1.5662 | 1.1323 |
| | Latitude (L) | | Sweden | | | | 0 | . |
| | Food (F) | | | high | | | 5.6592 | 1.1971 |
| | Food (F) | | | low | | | 0 | . |
| | Pesticide (P) | | | | control | | 6.1311 | 1.1525 |
| | Pesticide (P) | | | | 16 days | | 2.4749 | 1.1937 |
| | Pesticide (P) | | | | complete F0 | | 0 | . |
| | Heat (H) | | | | | 20°C | 1.2363 | 1.2215 |
| | Heat (H) | | | | | 30°C | 0 | . |
| | L * F | | France | high | | | -4.5605 | 1.5807 |
| | L * F | | France | low | | | 0 | . |
| | L * F | | Sweden | high | | | 0 | . |
| | L * F | | Sweden | low | | | 0 | . |
| | L * P | | France | | control | | -2.8720 | 1.5438 |
| | L * P | | France | | 16 days | | 0.09912 | 1.5739 |

| | | | | | | |
|---|---|---|---|---|---|---|
| L * P | France | | complete F0 | | 0 | . |
| L * P | Sweden | | control | | 0 | . |
| L * P | Sweden | | 16 days | | 0 | . |
| L * P | Sweden | | complete F0 | | 0 | . |
| L * H | France | | | 20°C | 1.5497 | 1.0996 |
| L * H | France | | | 30°C | 0 | . |
| L * H | Sweden | | | 20°C | 0 | . |
| L * H | Sweden | | | 30°C | 0 | . |
| F * P | | high | control | | -3.2476 | 1.6356 |
| F * P | | high | 16 days | | -1.0470 | 1.6962 |
| F * P | | high | complete F0 | | 0 | . |
| F * P | | low | control | | 0 | . |
| F * P | | low | 16 days | | 0 | . |
| F * P | | low | complete F0 | | 0 | . |
| F * H | | high | | 20°C | -1.1940 | 1.2679 |
| F * H | | high | | 30°C | 0 | . |
| F * H | | low | | 20°C | 0 | . |
| F * H | | low | | 30°C | 0 | . |
| P * H | | | control | 20°C | 1.0930 | 1.6860 |
| P * H | | | control | 30°C | 0 | . |
| P * H | | | 16 days | 20°C | 2.6207 | 1.7311 |
| P * H | | | 16 days | 30°C | 0 | . |
| P * H | | | complete F0 | 20°C | 0 | . |
| P * H | | | complete F0 | 30°C | 0 | . |
| L * F * P | France | high | control | | 1.8043 | 2.2065 |
| L * F * P | France | high | 16 days | | -0.4585 | 2.2499 |
| L * F * P | France | high | complete F0 | | 0 | . |
| L * F * P | France | low | control | | 0 | . |
| L * F * P | France | low | 16 days | | 0 | . |
| L * F * P | France | low | complete F0 | | 0 | . |

| | | | | | | |
|---|---|---|---|---|---|---|
| L * F * P | Sweden | high | control | | 0 | . |
| L * F * P | Sweden | high | 16 days | | 0 | . |
| L * F * P | Sweden | high | complete F0 | | 0 | . |
| L * F * P | Sweden | low | control | | 0 | . |
| L * F * P | Sweden | low | 16 days | | 0 | . |
| L * F * P | Sweden | low | complete F0 | | 0 | . |
| L * F * H | France | high | | 20°C | 0.1803 | 2.2974 |
| L * F * H | France | high | | 30°C | 0 | . |
| L * F * H | France | low | | 20°C | 0 | . |
| L * F * H | France | low | | 30°C | 0 | . |
| L * F * H | Sweden | high | | 20°C | 0 | . |
| L * F * H | Sweden | high | | 30°C | 0 | . |
| L * F * H | Sweden | low | | 20°C | 0 | . |
| L * F * H | Sweden | low | | 30°C | 0 | . |
| L * P * H | France | | control | 20°C | -4.0184 | 2.2220 |
| L * P * H | France | | control | 30°C | 0 | . |
| L * P * H | France | | 16 days | 20°C | 0.6042 | 2.2646 |
| L * P * H | France | | 16 days | 30°C | 0 | . |
| L * P * H | France | | complete F0 | 20°C | 0 | . |
| L * P * H | France | | complete F0 | 30°C | 0 | . |
| L * P * H | Sweden | | control | 20°C | 0 | . |
| L * P * H | Sweden | | control | 30°C | 0 | . |
| L * P * H | Sweden | | 16 days | 20°C | 0 | . |
| L * P * H | Sweden | | 16 days | 30°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 20°C | 0 | . |
| L * P * H | Sweden | | complete F0 | 30°C | 0 | . |
| F * P * H | | high | control | 20°C | 1.779 | 2.4002 |
| F * P * H | | high | control | 30°C | 0 | . |
| F * P * H | | high | 16 days | 20°C | 4.7315 | 2.4501 |
| F * P * H | | high | 16 days | 30°C | 0 | . |

| | | | | | | |
|---|---|---|---|---|---|---|
| F * P * H | | high | complete F0 | 20°C | 0 | . |
| F * P * H | | high | complete F0 | 30°C | 0 | . |
| F * P * H | | low | control | 20°C | 0 | . |
| F * P * H | | low | control | 30°C | 0 | . |
| F * P * H | | low | 16 days | 20°C | 0 | . |
| F * P * H | | low | 16 days | 30°C | 0 | . |
| F * P * H | | low | complete F0 | 20°C | 0 | . |
| F * P * H | | low | complete F0 | 30°C | 0 | . |
| L * F * P * H | France | high | control | 20°C | 3.6195 | 3.1668 |
| L * F * P * H | France | high | control | 30°C | 0 | . |
| L * F * P * H | France | high | 16 days | 20°C | -2.6111 | 3.2178 |
| L * F * P * H | France | high | 16 days | 30°C | 0 | . |
| L * F * P * H | France | high | complete F0 | 20°C | 0 | . |
| L * F * P * H | France | high | complete F0 | 30°C | 0 | . |
| L * F * P * H | France | low | control | 20°C | 0 | . |
| L * F * P * H | France | low | control | 30°C | 0 | . |
| L * F * P * H | France | low | 16 days | 20°C | 0 | . |
| L * F * P * H | France | low | 16 days | 30°C | 0 | . |
| L * F * P * H | France | low | complete F0 | 20°C | 0 | . |
| L * F * P * H | France | low | complete F0 | 30°C | 0 | . |
| L * F * P * H | Sweden | high | control | 20°C | 0 | . |
| L * F * P * H | Sweden | high | control | 30°C | 0 | . |
| L * F * P * H | Sweden | high | 16 days | 20°C | 0 | . |
| L * F * P * H | Sweden | high | 16 days | 30°C | 0 | . |
| L * F * P * H | Sweden | high | complete F0 | 20°C | 0 | . |
| L * F * P * H | Sweden | high | complete F0 | 30°C | 0 | . |
| L * F * P * H | Sweden | low | control | 20°C | 0 | . |
| L * F * P * H | Sweden | low | control | 30°C | 0 | . |
| L * F * P * H | Sweden | low | 16 days | 20°C | 0 | . |
| L * F * P * H | Sweden | low | 16 days | 30°C | 0 | . |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | L * F * P * H | Sweden | low | complete F0 | 20°C | 0 | . |
| | L * F * P * H | Sweden | low | complete F0 | 30°C | 0 | . |

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714 Appendix 2. Full model results of the ANOVAs testing for effects of latitude, food level,
715 pesticide concentration and adult heat condition on the response variables under study.

| Response variable | Effect | df 1, df 2 | *F* | *P* |
|---|---|---|---|---|
| Survival | Latitude (L) | 1, 4 | 76.72 | < 0.001 |
| | Food (F) | 1, 792 | 0.41 | 0.52 |
| | Pesticide (P) | 2, 792 | 0.37 | 0.69 |
| | Heat (H) | 1, 792 | 0.24 | 0.62 |
| | L x F | 1, 792 | 0.15 | 0.70 |
| | L x P | 2, 792 | 0.32 | 0.72 |
| | L x H | 1, 792 | 0.060 | 0.80 |
| | F x P | 2, 792 | 1.69 | 0.19 |
| | F x H | 1, 792 | 0.29 | 0.59 |
| | P x H | 2, 792 | 0.93 | 0.39 |
| | L x F x P | 2, 792 | 1.45 | 0.24 |
| | L x F x H | 1, 792 | 2.20 | 0.14 |
| | L x P x H | 2, 792 | 1.06 | 0.35 |
| | F x P x H | 2, 792 | 0.70 | 0.50 |
| | L x F x P x H | 2, 792 | 0.14 | 0.87 |
| Flying ability | Sex | 1, 554 | 4.34 | 0.038 |
| | Latitude (L) | 1, 4 | 0.050 | 0.83 |
| | Food (F) | 1, 554 | 0.090 | 0.76 |
| | Pesticide (P) | 2, 554 | 0.33 | 0.72 |
| | Heat (H) | 1, 554 | 28.31 | < 0.001 |
| | L x F | 1, 554 | 0.34 | 0.56 |
| | L x P | 2, 554 | 0.070 | 0.94 |
| | L x H | 1, 554 | 0.35 | 0.56 |
| | F x P | 2, 554 | 0.78 | 0.46 |
| | F x H | 1, 554 | 0.90 | 0.34 |
| | P x H | 2, 554 | 1.19 | 0.25 |
| | L x F x P | 2, 554 | 0.80 | 0.45 |
| | L x F x H | 1, 554 | 0.32 | 0.57 |
| | L x P x H | 2, 554 | 1.19 | 0.25 |
| | F x P x H | 2, 554 | 1.00 | 0.37 |
| | L x F x P x H | 2, 554 | 0.44 | 0.64 |
| Development time | Sex | 1, 605 | 0.63 | 0.43 |
| | Latitude (L) | 1, 605 | 2288.39 | < 0.001 |
| | Food (F) | 1, 605 | 20.01 | < 0.001 |
| | Pesticide (P) | 2, 605 | 0.11 | 0.90 |
| | L x F | 1, 605 | 0.060 | 0.81 |
| | L x P | 2, 605 | 0.020 | 0.98 |
| | F x P | 2, 605 | 6.35 | 0.0019 |
| | L x F x P | 2, 605 | 4.32 | 0.014 |
| Dry mass | Sex | 1, 441 | 36.00 | < 0.001 |
| | Latitude (L) | 1, 5.85 | 12.82 | 0.012 |
| | Food (F) | 1, 441 | 115.87 | < 0.001 |
| | Pesticide (P) | 2, 441 | 2.47 | 0.086 |

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|---|---|---|---|---|
| | Heat (H) | 1, 442 | 3.17 | 0.076 |
| | L x F | 1, 441 | 18.32 | < 0.001 |
| | L x P | 2, 441 | 0.28 | 0.76 |
| | L x H | 1, 442 | 0.13 | 0.72 |
| | F x P | 2, 441 | 1.35 | 0.26 |
| | F x H | 1, 441 | 1.35 | 0.25 |
| | P x H | 2, 441 | 0.21 | 0.81 |
| | L x F x P | 2, 441 | 0.060 | 0.94 |
| | L x F x H | 1, 441 | 0.070 | 0.80 |
| | L x P x H | 2, 441 | 0.99 | 0.37 |
| | F x P x H | 2, 441 | 1.57 | 0.21 |
| | L x F x P x H | 2, 441 | 0.75 | 0.47 |
| Water content | Sex | 1, 441 | 7.08 | 0.0081 |
| | Latitude (L) | 1, 6.27 | 14.60 | 0.0080 |
| | Food (F) | 1, 439 | 10.51 | 0.0013 |
| | Pesticide (P) | 2, 439 | 3.11 | 0.046 |
| | Heat (H) | 1, 441 | 36.43 | < 0.001 |
| | L x F | 1, 439 | 10.47 | 0.0013 |
| | L x P | 2, 439 | 0.22 | 0.81 |
| | L x H | 1, 441 | 0.52 | 0.47 |
| | F x P | 2, 439 | 0.59 | 0.55 |
| | F x H | 1, 439 | 0.36 | 0.55 |
| | P x H | 2, 439 | 0.89 | 0.41 |
| | L x F x P | 2, 439 | 0.29 | 0.75 |
| | L x F x H | 1, 439 | 1.43 | 0.20 |
| | L x P x H | 2, 439 | 1.76 | 0.17 |
| | F x P x H | 2, 440 | 0.62 | 0.54 |
| | L x F x P x H | 2, 440 | 0.67 | 0.51 |
| Fat content | Sex | 1, 423 | 1.51 | 0.22 |
| | Latitude (L) | 1, 4.2 | 49.30 | 0.0018 |
| | Food (F) | 1, 419 | 130.37 | < 0.001 |
| | Pesticide (P) | 2, 421 | 2.75 | 0.065 |
| | Heat (H) | 1, 423 | 28.38 | < 0.001 |
| | L x F | 1, 419 | 2.40 | 0.12 |
| | L x P | 2, 421 | 0.010 | 0.99 |
| | L x H | 1, 423 | 2.11 | 0.15 |
| | F x P | 2, 420 | 3.48 | 0.062 |
| | F x H | 1, 420 | 1.32 | 0.25 |
| | P x H | 2, 422 | 2.36 | 0.076 |
| | L x F x P | 2, 420 | 1.60 | 0.20 |
| | L x F x H | 1, 420 | 0.34 | 0.56 |
| | L x P x H | 2, 421 | 1.40 | 0.25 |
| | F x P x H | 2, 424 | 0.10 | 0.90 |
| | L x F x P x H | 2; 424 | 0.010 | 0.99 |
| Hsp70 levels | Sex | 1, 287 | 2.63 | 0.11 |
| | Latitude (L) | 1, 287 | 231.15 | < 0.001 |
| | Food (F) | 1, 287 | 114.86 | < 0.001 |

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|---|---|---|---|---|
| | Pesticide (P) | 2, 287 | 21.29 | < 0.001 |
| | Heat (H) | 1, 287 | 50.07 | < 0.001 |
| | L x F | 1, 287 | 4.09 | 0.44 |
| | L x P | 2, 287 | 1.39 | 0.25 |
| | L x H | 1, 287 | 11.59 | < 0.001 |
| | F x P | 2, 287 | 4.85 | 0.0085 |
| | F x H | 1, 287 | 8.84 | 0.0032 |
| | P x H | 2, 287 | 3.20 | 0.042 |
| | L x F x P | 2, 287 | 2.47 | 0.083 |
| | L x F x H | 1, 287 | 0.43 | 0.51 |
| | L x P x H | 2, 287 | 1.68 | 0.21 |
| | F x P x H | 2, 287 | 0.39 | 0.69 |
| | L x F x P x H | 2, 287 | 0.33 | 0.72 |
| PO activity | Protein | 1, 444 | 3.75 | 0.054 |
| | Mass | 1, 444 | 0.42 | 0.52 |
| | Sex | 1, 444 | 0.080 | 0.78 |
| | Latitude (L) | 1, 444 | 107.74 | < 0.001 |
| | Food (F) | 1, 444 | 52.48 | < 0.001 |
| | Pesticide (P) | 2, 444 | 57.99 | < 0.001 |
| | Heat (H) | 1, 444 | 1.03 | 0.31 |
| | L x F | 1, 444 | 1.30 | 0.26 |
| | L x P | 2, 444 | 1.10 | 0.29 |
| | L x H | 1, 444 | 34.63 | < 0.001 |
| | F x P | 2, 444 | 1.40 | 0.25 |
| | F x H | 1, 444 | 20.53 | < 0.001 |
| | P x H | 2, 444 | 1.84 | 0.17 |
| | L x F x P | 2, 444 | 6.14 | 0.0023 |
| | L x F x H | 1, 444 | 0.16 | 0.69 |
| | L x P x H | 2, 444 | 1.03 | 0.36 |
| | F x P x H | 2, 444 | 3.37 | 0.035 |
| | L x F x P x H | 2, 444 | 1.98 | 0.14 |

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718 Appendix 3. Means and standard error for each of the treatment combinations per response
719 variable.

| Response variable | Latitude | Food | Pesticide | Heat | Mean | Standard error | N |
|---|---|---|---|---|---|---|---|
| Development time | France | high | control | 20°C | 21.46 | 1.33 | 69 |
| | France | high | 16 days | 20°C | 21.71 | 1.32 | 68 |
| | France | high | complete F0 | 20°C | 22.24 | 1.34 | 67 |
| | France | low | control | 20°C | 26.59 | 1.32 | 67 |
| | France | low | 16 days | 20°C | 25.87 | 1.32 | 69 |
| | France | low | complete F0 | 20°C | 25.88 | 1.31 | 70 |
| | Sweden | high | control | 20°C | 62.91 | 1.71 | 63 |
| | Sweden | high | 16 days | 20°C | 64.29 | 1.73 | 60 |
| | Sweden | high | complete F0 | 20°C | 69.96 | 1.86 | 60 |
| | Sweden | low | control | 20°C | 72.87 | 1.78 | 57 |
| | Sweden | low | 16 days | 20°C | 70.15 | 1.89 | 51 |
| | Sweden | low | complete F0 | 20°C | 65.73 | 1.86 | 58 |
| Dry mass | France | high | control | 20°C | 2.76 | 0.11 | 23 |
| | France | high | control | 30°C | 2.84 | 0.12 | 21 |
| | France | high | 16 days | 20°C | 2.70 | 0.11 | 21 |
| | France | high | 16 days | 30°C | 2.67 | 0.12 | 20 |
| | France | high | complete F0 | 20°C | 2.74 | 0.11 | 21 |
| | France | high | complete F0 | 30°C | 2.64 | 0.12 | 21 |
| | France | low | control | 20°C | 2.44 | 0.11 | 23 |
| | France | low | control | 30°C | 2.26 | 0.11 | 21 |
| | France | low | 16 days | 20°C | 2.34 | 0.11 | 21 |
| | France | low | 16 days | 30°C | 2.30 | 0.11 | 21 |
| | France | low | complete F0 | 20°C | 2.31 | 0.11 | 21 |
| | France | low | complete F0 | 30°C | 2.24 | 0.11 | 22 |
| | Sweden | high | control | 20°C | 3.16 | 0.12 | 16 |
| | Sweden | high | control | 30°C | 3.15 | 0.12 | 19 |
| | Sweden | high | 16 days | 20°C | 3.13 | 0.12 | 18 |
| | Sweden | high | 16 days | 30°C | 2.99 | 0.12 | 16 |
| | Sweden | high | complete F0 | 20°C | 3.15 | 0.12 | 13 |
| | Sweden | high | complete F0 | 30°C | 3.05 | 0.12 | 15 |
| | Sweden | low | control | 20°C | 3.00 | 0.12 | 17 |
| | Sweden | low | control | 30°C | 2.87 | 0.12 | 17 |
| | Sweden | low | 16 days | 20°C | 2.97 | 0.12 | 16 |
| | Sweden | low | 16 days | 30°C | 2.92 | 0.12 | 15 |
| | Sweden | low | complete F0 | 20°C | 2.91 | 0.12 | 14 |
| | Sweden | low | complete F0 | 30°C | 2.89 | 0.12 | 17 |
| Water content | France | high | control | 20°C | 89.04 | 0.17 | 23 |
| | France | high | control | 30°C | 88.42 | 0.17 | 20 |
| | France | high | 16 days | 20°C | 89.00 | 0.17 | 21 |
| | France | high | 16 days | 30°C | 88.57 | 0.17 | 20 |
| | France | high | complete F0 | 20°C | 89.07 | 0.17 | 20 |
| | France | high | complete F0 | 30°C | 88.75 | 0.17 | 21 |
| | France | low | control | 20°C | 89.30 | 0.17 | 23 |
| | France | low | control | 30°C | 89.07 | 0.17 | 21 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | France | low | 16 days | 20°C | 89.27 | 0.17 | 21 |
| | France | low | 16 days | 30°C | 89.12 | 0.17 | 21 |
| | France | low | complete F0 | 20°C | 89.48 | 0.17 | 21 |
| | France | low | complete F0 | 30°C | 89.15 | 0.17 | 22 |
| | Sweden | high | control | 20°C | 88.48 | 0.19 | 16 |
| | Sweden | high | control | 30°C | 88.22 | 0.18 | 19 |
| | Sweden | high | 16 days | 20°C | 88.66 | 0.19 | 17 |
| | Sweden | high | 16 days | 30°C | 88.28 | 0.19 | 16 |
| | Sweden | high | complete F0 | 20°C | 88.72 | 0.21 | 13 |
| | Sweden | high | complete F0 | 30°C | 88.59 | 0.20 | 15 |
| | Sweden | low | control | 20°C | 88.73 | 0.20 | 16 |
| | Sweden | low | control | 30°C | 88.30 | 0.19 | 17 |
| | Sweden | low | 16 days | 20°C | 88.90 | 0.20 | 15 |
| | Sweden | low | 16 days | 30°C | 87.80 | 0.20 | 15 |
| | Sweden | low | complete F0 | 20°C | 88.81 | 0.20 | 14 |
| | Sweden | low | complete F0 | 30°C | 88.42 | 0.19 | 17 |
| Fat content | France | high | control | 20°C | 9.89 | 0.25 | 22 |
| | France | high | control | 30°C | 8.96 | 0.26 | 21 |
| | France | high | 16 days | 20°C | 9.00 | 0.26 | 21 |
| | France | high | 16 days | 30°C | 8.52 | 0.26 | 20 |
| | France | high | complete F0 | 20°C | 9.12 | 0.26 | 21 |
| | France | high | complete F0 | 30°C | 8.44 | 0.26 | 21 |
| | France | low | control | 20°C | 8.15 | 0.25 | 23 |
| | France | low | control | 30°C | 7.16 | 0.26 | 21 |
| | France | low | 16 days | 20°C | 7.94 | 0.26 | 21 |
| | France | low | 16 days | 30°C | 6.99 | 0.26 | 21 |
| | France | low | complete F0 | 20°C | 7.87 | 0.25 | 21 |
| | France | low | complete F0 | 30°C | 6.86 | 0.25 | 22 |
| | Sweden | high | control | 20°C | 10.51 | 0.29 | 16 |
| | Sweden | high | control | 30°C | 9.80 | 0.28 | 17 |
| | Sweden | high | 16 days | 20°C | 10.46 | 0.28 | 18 |
| | Sweden | high | 16 days | 30°C | 9.55 | 0.30 | 15 |
| | Sweden | high | complete F0 | 20°C | 9.74 | 0.35 | 10 |
| | Sweden | high | complete F0 | 30°C | 9.59 | 0.37 | 11 |
| | Sweden | low | control | 20°C | 9.60 | 0.29 | 16 |
| | Sweden | low | control | 30°C | 8.88 | 0.32 | 13 |
| | Sweden | low | 16 days | 20°C | 9.06 | 0.30 | 15 |
| | Sweden | low | 16 days | 30°C | 7.98 | 0.31 | 14 |
| | Sweden | low | complete F0 | 20°C | 8.83 | 0.31 | 14 |
| | Sweden | low | complete F0 | 30°C | 8.73 | 0.30 | 15 |
| Hsp70 levels | France | high | control | 20°C | 7.99 | 0.23 | 12 |
| | France | high | control | 30°C | 8.65 | 0.24 | 11 |
| | France | high | 16 days | 20°C | 8.66 | 0.23 | 12 |
| | France | high | 16 days | 30°C | 9.07 | 0.24 | 12 |
| | France | high | complete F0 | 20°C | 8.57 | 0.23 | 12 |
| | France | high | complete F0 | 30°C | 9.21 | 0.22 | 13 |
| | France | low | control | 20°C | 7.49 | 0.24 | 11 |
| | France | low | control | 30°C | 7.69 | 0.23 | 12 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | France | low | 16 days | 20°C | 7.99 | 0.22 | 13 |
| | France | low | 16 days | 30°C | 7.98 | 0.23 | 12 |
| | France | low | complete F0 | 20°C | 7.93 | 0.23 | 12 |
| | France | low | complete F0 | 30°C | 8.13 | 0.23 | 12 |
| | Sweden | high | control | 20°C | 8.40 | 0.23 | 12 |
| | Sweden | high | control | 30°C | 10.45 | 0.22 | 13 |
| | Sweden | high | 16 days | 20°C | 10.38 | 0.22 | 13 |
| | Sweden | high | 16 days | 30°C | 11.40 | 0.24 | 12 |
| | Sweden | high | complete F0 | 20°C | 10.17 | 0.24 | 12 |
| | Sweden | high | complete F0 | 30°C | 11.13 | 0.22 | 13 |
| | Sweden | low | control | 20°C | 8.35 | 0.24 | 11 |
| | Sweden | low | control | 30°C | 9.48 | 0.23 | 12 |
| | Sweden | low | 16 days | 20°C | 8.70 | 0.23 | 12 |
| | Sweden | low | 16 days | 30°C | 9.49 | 0.23 | 12 |
| | Sweden | low | complete F0 | 20°C | 9.32 | 0.22 | 13 |
| | Sweden | low | complete F0 | 30°C | 9.36 | 0.24 | 11 |
| PO activity | France | high | control | 20°C | 15.02 | 0.70 | 23 |
| | France | high | control | 30°C | 10.87 | 0.76 | 21 |
| | France | high | 16 days | 20°C | 11.71 | 0.74 | 21 |
| | France | high | 16 days | 30°C | 10.12 | 0.76 | 20 |
| | France | high | complete F0 | 20°C | 10.53 | 0.74 | 21 |
| | France | high | complete F0 | 30°C | 9.06 | 0.76 | 21 |
| | France | low | control | 20°C | 11.88 | 0.71 | 23 |
| | France | low | control | 30°C | 11.22 | 0.77 | 21 |
| | France | low | 16 days | 20°C | 11.30 | 0.75 | 21 |
| | France | low | 16 days | 30°C | 10.53 | 0.77 | 21 |
| | France | low | complete F0 | 20°C | 10.74 | 0.75 | 21 |
| | France | low | complete F0 | 30°C | 7.96 | 0.75 | 22 |
| | Sweden | high | control | 20°C | 20.88 | 0.88 | 16 |
| | Sweden | high | control | 30°C | 18.07 | 0.79 | 19 |
| | Sweden | high | 16 days | 20°C | 18.47 | 0.85 | 18 |
| | Sweden | high | 16 days | 30°C | 16.61 | 0.86 | 16 |
| | Sweden | high | complete F0 | 20°C | 15.18 | 0.89 | 13 |
| | Sweden | high | complete F0 | 30°C | 14.93 | 0.82 | 15 |
| | Sweden | low | control | 20°C | 15.78 | 0.86 | 17 |
| | Sweden | low | control | 30°C | 18.65 | 0.82 | 17 |
| | Sweden | low | 16 days | 20°C | 12.00 | 0.89 | 16 |
| | Sweden | low | 16 days | 30°C | 10.61 | 0.88 | 15 |
| | Sweden | low | complete F0 | 20°C | 10.76 | 0.91 | 14 |
| | Sweden | low | complete F0 | 30°C | 9.52 | 0.82 | 17 |

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